BIO- EFFICACY OF *BACILLUS THURINGIENSIS* BERLINER AGAINST *DIACRISIA OBLIQUA* WALKER (LEPIDOPTERA : ARCTIIDAE)

A

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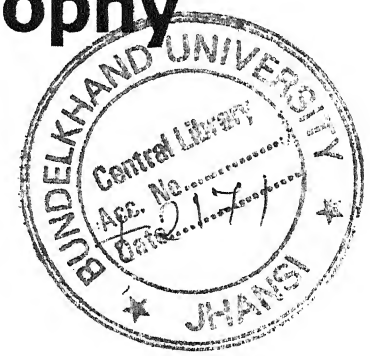

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Certificate

This is to certify that the work embodied in this thesis, entitled "BIO – EFFICACY OF BACILLUS THURINGIENSIS BERLINER AGAINST DIACRISIA OBLIQUA WALKER (LEPIDOPTERA : ARCTIIDAE)" in Zoology from Bundelkhand University, Jhansi has been carried out by Mr. Shatrughan Singh under my supervision in Department of Zoology, D. V. (P.G.) College, Orai (Jalaun). He has fulfilled all the requirements for the degree and has worked more than 200 days in the department commencing from the date of application for registration in Bundelkhand University, Jhansi (U.P.).

The work included in thesis is original unless otherwise stated and has not been submitted for any degree anywhere.

3/10/ 2006

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CHAPTER ONE

CHAPTER - ONE

INTRODUCTION:

Bihar hairy caterpillar, *Diacrisia obliqua* Walker (Arctiidae : Lepidoptera), a notorious polyphagous pest widely distributed in India, Bangladesh, Ceylon, China, Pakistan, Nigeria, Burma etc., had been infesting pulses, oil seed, fibre crops, vegetables and fruit plants at Orai (Jalaun) and surrounding areas but it never caused anxiety in farmers as its damage was always below economic injury level but from 2000 onwards, with the increased irrigation facilities which introduced changes in cropping pattern in crops, the population of *D. obliqua* started growing with a considerable speed and suddenly reached an explosive stage in September, 2003. There was hardly any crop field not infested heavily by this pest at that time. It was heavy and unusual out break, caterpillars in large number were found roaming on roads and in houses close to farm lands. Infact, farmers were scared by the sudden eruption of population of this pest.

Under the infestation referred to as above, the crops were badly defoliated and it was feared that these crops might be exterminated due to ravage of the pest if the situation was not controlled immediately. To save the crops from this pest, farmers used already recommended powerful insecticides such as BHC, parathion, malathion, endosulfan and other synthetic insecticides

but surprisingly, the older larvae survived their toxicity. This demanded trial with more powerful insecticides which would have controlled the abnoxious pest like an avenging angel since the use of such insecticides have given spectacular results in many cases but high hopes raised by their introduction has not been fully achieved.

Economic entomologists had made good efforts in search of such chemicals and consequently they have been successful in tracing a good number of chemical insecticides to be employed as an alternative control strategy against *D. obliqua*. But chemical insecticides may result in acute and long term effects including sickness and death of people, and useful animals, development of resistant population, environmental pollution, undesirable side effects on non-target animals and plants and disruptive impacts on ecosystem. Such problems forced the economic entomologists to proceed further in search of safer methods of pest management.

With the growing realization of hazards and side ill effects connected with the indiscriminate use of insecticides, entomologists have developed a new concept of pest control and termed as Integrated Pest Management (I.P.M.) which refers to a system that utilizes all suitable techniques and methods in as compatible manner as possible and maintain the pest population at levels below those causing injury to our crops and economy (Mathur and Prem Kishore, 1987).

The I.P.M. system does not exclude chemical insecticides from the strategy of pest management, but it advocates its judicious use for combating the pest population in addition to other methods of insect control. In this context, the role of bio-control agents viz. parasites, predators and microbes need no emphasis due to their specificity, effectiveness, safety to nontargeted organisms including pollinators and natural enemies. It would be worthy for mention here that most of the biological agents are compatible with other methods of pest control.

In recent years, entomologists are leaning their attention on the exploration and exploitation of microbial agents including virus, bacteria and fungi for the pest suppression. Interestingly, few have been widely tested and proved very effective against pernicious pests of agricultural crops. In certain developed countries a number of bio-pesticides have been registered for field application on various vegetables, fruits and other crops of agricultural, horticultural and forest importance.

One of these approaches which has captured world wide attention is the development of compounds acting selectively on some groups of insects by inhibiting or enhancing the activity of biochemical sites such as respiration (diafenthiuron), the nicotinyl acetyl chlorine receptor (imidacloprid), the salivary glands of sucking pests (pymetrozine). Progress has been made to introduce improved bio-control agents such as *Bacillus thuringiensis (B.t.)* for controlling lepidopteran, coleopteran and dipteran pests. *B. thuringiensis* kills insects primarily through the action of δ-endotoxins, a proteinous constituent produced

during sporulation; it affects the insect mid gut epithelium upon ingestion (Hofte and Whiteley, 1989; Gill *et al.*, 1992 ; Navon, 1993). Most conventional *B.t.* products are based on the subspecies *kurstaki* HD – 1 introduced by Abbott in laboratory, followed by Sandoz, Nova, Ecogen and Monsato for controlling lepidopteran pests (Navon, 1993).

Bacillus thuringiensis (*B.t.*) is an aerobic, gram positive, spore forming bacterium found in the environment. It produces a number of insect toxins, the most distinctive of which is a protein crystal formed during sporulation (Bulla *et al.*, 1977; Whiteley and Schnepf, 1986). It is a crystalline protein inclusion, that is the principal active ingredient in formulations currently in use. Although *B.t.* was described by Berliner in 1911 and its potential as an insecticide was recognized in early 1980's. Now a days various formulations of *B.t. kurstaki* such as Dipel (HD-1,Abbot), Thuricide (HD-1, Sandoz), Biobit (HD-1, Nova), Javelin (NRD 12, Sandoz and MVP (monsanto) are available for controlling the lepidopteran pests. These insecticides are considered safe to the environment and natural enemies. Hence, they are considered important components in IPM programs.

Heimpel (1967) observed the toxicity of the pathogen and found it depends upon parasporal bodies which contain toxins. These toxins are known as α, β and y – exotoxin and δ – endotoxin. Dulmage *et al.* (1981) reported more than twenty varieties of *B. thuringiensis* which differ from each other by the presence of endospore and exotoxin crystals. Although *B. t.* contains four toxins,

yet, more emphasis has been given only to the crystalline endotoxin because of its high toxic activity against lepidopteran pests.

Because of the great diversity of *B.t.* toxins occurring in nature, it has been suggested that these toxins might be used in mixtures and rotations to delay pest adaptation. The compatibility of *Bt.* with many chemical insecticides warrants the study to explore its feasibility under IPM system. Generally, small amount of insecticide added to *B.t.* suspension reduces the pest population and also minimizes the high cost of application of these biotic agents. As per literature available, it is pathogenic to more than 525 insect species belonging to different orders mainly to lepidoptera, diptera, hymenoptera and coleoptera (Sundrababu, 1985). Ramakrishnan and Kumar (1977) reported 170 species of lepidopterous larvae which were pathogenic to *B. thuringiensis* .

A perusal of literature divulge that work done in India related to *B.t.* is very scattered and fragmentary. Most of the work done in India under laboratory and field conditions is concerned with pathogenicity tests. However, there are other important aspects like effects of exposure period on insect mortality, effect of lethal and sublethal concentrations of *B.t.* on the development of insects, efficacy of different commercial products and their combinations with different insecticides against the particular insect species, which have not been paid due attention so far.

Considering the aforesaid facts in view, following studies have been planned against *D. obliqua* under laboratory conditions.

(a) Effect of different formulations of *B.t.* on growth of *D. obliqua.*

(b) Effect of different formulations of *B.t.* on development of *D. obliqua*.

(c) Effect of different formulations of *B.t.* on fecundity and fertility of *D. obliqua*

(d) Sex specific sterility of different formulations on sexes.

(e) Compatibility of Dipel with certain commonly used chemical insecticides.

The results, thus, obtained have been discussed critically in the light of earlier findings in the present thesis.

CHAPTER TWO

REVIEW OF LITERATURE:

A detailed exhaustive and systematic study of literature has critically been done on various aspects of the present research work with special reference to the on the effect of *Bacillus thuringiensis* on growth, longevity and reproduction of *D. obliqua* under laboratory conditions. Its compatibility with common insecticides also studied, Sincere attempts have also been made to cite all the relevant references related with the present investigation.

Bacillus thuringiensis is an aerobic, gram positive, spore forming bacterium found rather commonly in the environment. It produces a number of insect toxins, the most distinctive of which is a protein crystal formed during sporulation (Hannay and Fitz – James, 1995; Bulla *et.al.* 1977; Whiteley & Schnepf, 1986). It is this crystalline protein inclusion, that is the principal active ingredient in formulations currently in use. During the early 1980s it was found that *B.t.* was amenable to genetic manipulation using recombinant DNA techniques because the genes that encode for δ-endotoxin production are borne on plasmids (Faust *et.al.*, 1983). The gene transfer technology progressed rapidly and biotechnological developments hold promise for facilitating genetic improvements in the potency and host spectrum of *B.t.* strains (Carlton, 1988; Martens *et.al.*, 1990)

Since *B.t.* resistance is an evolutionary phenomenon, it is controlled by four factors : (i) natural variation ; (2) *B.t.*. selection; (3) survival of selected individuals and (4) reproduction of *Bt.* selected survivors. Natural variation to *B.t.* within field populations of pests appears to be quite high (Kennedy and Whalon, 1995).

Angus (1956) observed that the exposure of larvae of *B. mori* to *B. thuringiensis* for twenty four hours caused mortality within three days.

Jaqves and Fox (1960) studied the comparative toxicity of two varieties of *B. thuringiensis* against *Pieris rapae* and found variety *entomocidus* was more effective than variety *berliner.*

Heimpel (1961) tested two varieties of *B. thuringiensis* against four species of saw fly and noted low virulence of the pathogen against them; variety *thuringiensis* and *sotto* were noted equally toxic against *Pristiphora erichsonii.*

Herfs (1963) studied the toxicity of six varieties of *B. thuringiensis* against *P. brassicae & Lymantria dispar* and observed the following descending order to their toxicity. Varieties *galleriae alesti> dendrolimus> thuringiensis> euxoae> sotto* against *P. brassicae* and variety *dendrolimus> galleriae> euxoae> thuringiensis> alesti> sotto* against *L.dispar.*

Smirnoff (1965) tested the efficacy of different strains of *B. thuringiensis* viz; *thuringiensis, dendrolimus, alesti, entomocidus, sotto & B. cereus against Choristoneura fumiferana* and reported that these strains registered 96, 91.3, 82.4, 86.2, 76.2 and 70.7 per cent control, respectively.

Kulshrestha and co-workers (1965) carried out the experiment to observe the toxicity of Thuricide against third to sixth larval instars of castor semilooper and the investigators did not notice any difference in mortality against third and fourth instar larvae. However, mortality did differ in fifth and six instar larvae of *A. janata;* higher mortality was recorded in fifth instar larvae when compared with the sixth instar.

Ruperez (1966) studied rapid mortality of 4^{th} instar larvae of *Thaumetopoea pitvocampa* Boln. due to variety *entomocidus* (100% in 18 days) as compared to variety *galleriae* (100% in 26 days).

Broersma and Buxton (1967) tested the effect of six strains of *B. thuringiensis* on mortality and pathological effects against cabbage looper, *Trichoplusia ni.* The highest pathogenicity and mortality was shown by *galleriae* followed by *B. thuringiensis, sotto & alesti.* The *Bacillus* strain like *entomocidus* and *finitimus* caused the lowest mortality and pathogenicity in *T. ni.*

Angus and Norris (1968) observed that two isolates out of 13 isolates tested, did not prove toxic upto the dose of 100 μ/gram/ to larvae of *Bombyx mori.* LD_{50} of *entomocidus, sotto* and *thuringiensis* was found 0.09, 0.28 and 10 μ / gm of larvae, respectively.

Yamurias and Angus (1969) reported that the variety *berliner* was more toxic when compared with *sotto* against *Tineola biselliela* larvae. The same authors in 1970 tested pathogenicity of 10 strains of *B. thuringiensis* against spruce bud worm, (*Choristoneura fumiferana)* and reported that strains namely:

alesti, berliner, entomocidus, sotto & galleriae were moderately pathogenic, *kenyae* and *dendrolimus* less and *finitimus* was slightly pathogenic.

Roosmoore *et.al.* (1970) tested isolates against *Hemorocampa leucostigma* and found that the isolates in the following descending order of toxicity *B. thuringiensis* > *sotto*> *entomocidus* > *aizawai.*

Abdllah and Abdul Nasar (1970) studied the exposure of larvae to sub-lethal concentration of *B. thuringiensis* and found that the larval period of S. *littoralis* increased considerably.

Benz (1971) reported an excellent review on compatibility of chemical pesticides with *B. thuringiensis* and found that most of the insecticides are compatible with the bacterium having little or no effect on spore germination or cell multiplication. He further added that low concentration of carbamate and organophosphates did not effect bacterial growth rather in certain cases improved it, whereas chlorinated hydrocarbons inhibited the growth of tested pathogen.

Dulmage and Martinez (1973) observed that the constant exposure of *H. virescens* larvae to sub-lethal levels of endotoxin retarded the development with reduction in larval weight, pupation and adult emergence. They also observed that the constant exposure of insect larvae even to sublethal concentration of *B.t.* results in heavy mortality at prepupa and pupal stage of *H. virescens.*

Ridet (1973) observed the sensitivity of *Lymantria dispar* to different varieties of *B. thuringiensis*. In general, *galleriae, subtoxicus, entomocidus,* were the most effective in terms of mortality, whereas *berliner, alesti, sotto & dendrolimus* resulted in low rate of mortality of *L.dispar.*

Creighton and co-workers (1973) studied the effect of carbaryl and chlordimeform in combination of the bacterium (Dipel var. *alesti*)against *H.zea.* They observed an additive effect in combination with these chemical pesticides.

Govindrajan *et. al.* (1975) reported that *B. thuringiensis* causes mortality in first instar larvae of *Spodoptera litura* within 7.3 days after infection, whereas second to sixth instar larvae were unaffected by this treatment.

Creighton and McFadden (1975) reported that Dipel *(kurstaki)* 3.2% W.P. was more effective in reducing plant damage than flowable powder or W.P. formulation of *Bactospeine (B. thuringiensis),* insect pest controlled by them were *T.ni. P.rapae* & *P. xylostella.*

Narayanan and Jayraj (1975) observed that *B.t.* causing cessation of feeding in larvae of *P. demolius* which resulted in the reduction of size, weight and lipid content with decrease in body length and weight to about 47.8 and 46.1 per cent, respectively.

Dobrivojevic and Injac (1975) tested Dipel (*kurstaki*) and Bactospeine (*B. thuringiensis*) @ 0.5, 0.125 and 0.031 per cent against wooly moth and reported it to be the most effective with LD_{50} of 11 ppm as compared with LD_{50} of 18 ppm in Bactospeine.

Svestka (1975) found Dipel as the most effective treatment against the larvae of *Operophtera brumata.* The per cent mortality recorded by Dipel was 93-100% which was followed by Thuricide (90-93%) and Entobakterin-3 (70 to 90%). In 1980, he reported that Permathrin in combination with *B. t.* caused additive response to the larvae of *O. brumata* and *Tortrix vividana.*

Creighton and McFadden (1975) reported that at least 1/16 lb/acre of chlordimeform hydrochloride in spray of *B. thuringiensis* is required for additive effect against *T. ni & P. xylostella.*

Hussain and Askari (1976) tested the combination of 19.4 BIU *B. thuringiensis* with 0.56 kg.azinphos-methyl per hectare against the larvae of *E. insulana,* but they did not find any increase in efficacy with regard to the chemical used alone.

On apple trees, the control of *Oryzia antigua* was achieved by the application of *B. thuringiensis* 'Dipel' (*kurstaki*) @0.05% which gave better control as compared to 0.1 to 0.3% Bactospeine (*B. thuringiensis*) and Selectizin (*aizawai*) (Lipal *et.al.* :1977).

Survlivelu *et.al.* (1977) observed that Biotrol at 1 g/lit reduced the pod borer infestation on field bean and it was next in order of toxicity to Endosulfan and Chlorpyriphos.

Hamilton and Attia (1977) reported an antagonistic effect of Dimethoate, when used with *B. thuringiensis* against *P. xylostella.*

Verma and Gill (1977) reported that Dipel (*Kurstaki*) @ 1 and 1.5 kg/lit. of water was more effective than *Bactospeine,* thuricide (*B.thuringiensis*) when used at the same concentration against *Plutella xylostella.*

Dulmage and co-workers (1978) studied a critical growth phase responsible for deformities in the insects. They observed such stages between hatch and the third instar of *H. virescens.* Once this stage passes, little difference occurs between treated and control larvae. They also experienced that the sub-lethal levels of endotoxin did not cause direct mortality of the host.

Jaques and Laing (1978) revealed that low dosage mixture of chlordimeform and *B. thuringiensis* controlled *T. ni & P.rapae* in much better way than used alone.

Pinnock and Milstead (1978) observed that field population of *Arachis orgyrospilus* larvae were virtually eliminated from road side plantation of red bud trees in California when the treatment with *B. thuringiensis* var. *kurstaki* at 192 and 80 x 10^6 IU/lit. took place partial control was *obtained* with *B. thuringiensis* var. *thuringiensis* @ 32.1x 10^6IU/lit.

Hamed (1978) studied the potency of different preparations of *B.thuringiensis* against *Yponomenta evonmellus & Y. padellus* and found it to be 1:1, 1:2.2, 1:1.3, 1:0.45 and 1:0.3 for'Dipel' (*kurstaki*), 'Thuricide (*kurstaki*), unrefined test preparation, partially refined test preparation, 'Biotrol' (*thuringiensis*), respectively.

Kumar and Jayraj (1978) evaluated the effectiveness of Biotrol x k at the concentration of 10, 20 and 30 g WP/lit. of water against the larvae of *P. ricini* and each concentraiton was found lethal against this insect.

Srivastava and Nayak (1978) studied the efficacy of four formulations of *B. thuringiensis* against *Cnaphalocrosis medinalis* and reported that all formulations were superior to control.

Yadav (1979) evaluated the efficacy of Thuricide HP (*B. thuringiensis var. kurstaki*) at various concentrations against 7 and 21 days old larvae of *Sesamia inferens* and found quite promising against it.

Triggiani (1979) tried *B. thuringiensis* var. *kurstaki* at the concentration of 100-400 g/lit. against the larvae (IInd – IIIrd instar) of *Prothetria dispar* and reported effective control (64-93%) of this insect.

Schmidt (1979) observed that the larvae of *Plodia interpunctella*, which survived after exposure to Thuricide at the concentration of 20 mg (64 x 10^3IU) dust/100 gm, resulted in the prolongation of larval duration by 3-4 weeks, but the adults, which emerged from intoxicated larvae were fertile.

Lencheva and Kuzmanova (1980) reported that the reduction in concentration by 90 per cent of the chemical insecticides in combination of *B. thuringiensis* against larvae of *Lycia hiritans* resulted in considerable increase in effectiveness of the pathogen var. *kurstaki* (Dipel) and *galleriae* (Entobacterin) except Phosalone which antagonised the effect of the pathogen.

Sogoyan and Slobodyanyuk (1980) observed that effectiveness of insecticide and *B. thuringiensis* mixture decreased with the increase in concentration of Carbaryl and Trichlorphon against *Galleria mellonella*,but the reverse occurred in case of *B. mori*. They also demonstrated that organochlorine compounds manifest the synergistic effect with *Bacillus*, whereas organophosphates tested reduce the toxicity of the bacterium.

Milstead and co-workers (1980) carried out a field trial with the commercial preparation of *B. thuringiensis* at the concentration as low as 0.06% (wt./vol.) against the tent caterpillar, *Melacosoma constrictum*,. and found the bacterium at this concentration quite effective to suppress the larval population of the insect species.

Salma *et.al.*(1981) studied on the effect of exposure time on the larvae of *S. littoralis* and *H. armigera*, subjected to different concentrations of *B. thuringiensis.* They revealed that at the higher concentration larval mortality was cent per cent irrespective of the exposure period (1-7days) and the LT_{50} was directly related with the exposure time.

Tiwari and Mehrotra (1981) observed the effectiveness of Bactospeine at various concentrations against *A. janata* and reported the LT_{50} inversely related with *B. thuringiensis* concentrations. They also studied the effect of *B. thuringiensis* on the pH of gut and haemolymph of *A. janata* and *S.litura* and found no change in pH of the haemolymph in fasting, treated and normal larvae.

Krishnaiah *et.al* (1981) reported that Dipel @ 0.05% effectively controlled pod borer of field bean, *Adisura atkinsoni*. They further noticed that *P. xylostella* could be efficaciously suppressed by Dipel if applied @0.5 kg/ha at weekly interval.

Smirnoff (1981) found that 3rd and 4th larval instars of *C.fumiferana* are more susceptible to *B. thuringiensis* than 5th and 6th larval instars. However, the first instar larvae were most susceptible to *B. thuringiensis*.

Salama *et.al.*(1981) studied the effect of sub-lethal levels on three species of insects *viz.*: *S. litura, S. exigua & H. armigera* and reported adverse effect on the adult emergence, fecundity in addition to the prolongation of developmental period and reduction in the pupal weight. However, longevity of adults remains uneffected with the larval treatment.

Sneh *et. al.* (1981) studied the effect of more than 50 isolates of *B. thuringiensis* against *Spodoptera littoralis* and out of these 7 isolates gave 100% mortality of 2nd instar larvae.

Deshpandey and Ramkrishnan (1982) reported that varieties *kurstaki, galleriae, berliner & sotto* were pathogenic to *Achoea janata,* but *entomocidus & sizawai* failed to respond.

Abdul Sattar and Watson (1982) reported that ability of *H. virescens* larvae to recover from the infection of *B. thuringiensis* decreases as the exposure time or dosage rate increases. They also reported on increase in larval and pupal

period of *H. virescens* after exposure to the bacterium. However, longevity and fecundity of adults and the viability of eggs remained unaffected.

Mc Gaughey (1982) tested dust and wettable preparations of *B. thuringiensis* against the Indian meal moth (*Plodia interpunctella*) and stated that the dust formulation of *B. thuringiensis* was superior to wettable powder.

Sareen *et.al.*(1983) evaluated the effect of *B. thuringiensis* on the larval duration and pupal weight of *S. litura*.

The experiment of Mohamed and co-workers (1983) with the mixture of *B. thuringiensis* and chlordimeform of cyhexatin revealed that it causes greater mortality of *H. virescens* when they were used alone.

Shekhar and Joshi (1984) tested Fenvelerate, Endosulfan, Quinalphos, Malathion and Carbaryl and their combination with *B. cereus* against *T.ni*. They reported that pyrethroids in combination with the *B. thuringiensis* increase the kill of *T.ni* by 2.5 times with the same dose of insecticides used alone.

Broza *et.al.* (1984) found that commercial application of var. *entomocidus* in cotton field., suppressed effectively the first and second instar larvae of *S.littoralis.*

Krieg *et.al.* (1984) conducted an experiment on the control of colorado beetle, *Leptinotarsa decemlineata by B. thuringiensis* var. *tenebrionis* at concentration of $5x10^{14}$ spores/ha and found the effective control of the beetle with the bacterium.

Fast and Dimond (1984) demonstrated a greater weight loss in larvae and pupae of *C. fumiferana,* when intoxicated with heavy dose of *B. thuringiensis.* They also studied the relationship between the larval instars of spruce bud worm, *C. fumiferana* and the *pathogenicity* of *B. thuringiensis*, but they did not find any significant difference in LC_{50} and LT_{50} values against fourth to sixth instar larvae of the *C. fumiferana.*

Fast and Regniere (1984) reported that the recovered larvae of spruce bud worm gained weight at the same rate as the untreated one, when exposed to *B. thuringiensis.* They also showed that the extension of exposure period from one day to continuous six days resulted in 500 times reduction in LC_{50} and equivalent reduction in LT_{50} of spruce budworm larvae.

Bosgelmen and co-workers (1984) tested different concentrations of Dipel, Tribactor ETB WP and Bactospeine against the larave of *Galleria melonella* and found each formulation effective against this pest.

Odak *et.al.*(1984) evaluated Thuricide and Bactospeine against *H. armigera* and recorded mortality ranging from 70 to 100 and 20-65 per cent, respectively.

Srivastava (1984) assessed the effectiveness of Dipel. Bactospeine and *B.thuringiensis* var. *alesti* against *Papilio demoleus* at the concentration of 0.01 0.025, 0.05, 0.125, 0.25 and 0.5 per cent. He also worked out the LC_{50} of Dipel, *B. thuringiensis* var. *alesti* and Bactospeine,which were 0.03112,0.05012 and 0.07930, respectively. The spore number required to give 50% kill in Dipel

was 75 x 10^7 spores, in *B. thuringiensis* var. alesti, 125 x 10^7 and in Bactospeine 21 x 10^4 spores. On the basis of spore number Bactospeine was considered to be the most effective followed by Dipel and *B. thuringiensis*. Var. *alesti.*

Holston and Hard (1985) observed that Dipel 4L & Thuricide 32 LB resulted in 69 and 76 per cent reduction in the population of *C. conflictana*, respectively.

Salama (1985) reported the adverse effect on the fecundity and longevity of *S. littoralis*, when the moths were directly fed on sucrose diet containing 17.2x10^3 IU of *B. thuringiensis* var. *galleriae.*

Yang and co-researchers (1985) studied the effect of *B. thuringiensis*. Var. *kurstaki* on the larvae of *S. litura*, found poor pupation followed by poor emergence of the adults, which finally did not lay eggs at all.

EL– Husseini and Afify (1985) tested the efficiency of *B. thuringiensis* containing 0.25% sugar, against 1st to 5th larval instar of *E. insulana.* They apprised the susceptibility of all the instars to this combination of pathogen.

Moawad *et.al.*(1985) studied the effectiveness of Bactospeine and Dipel powder against *Earias insulana* at the concentration of 0.05,0.1, 0.2 and 0.4 per cent. They asserted Dipel as more effective biopesticide than Bactospeine. The LC_{50} and LT_{50} values were 0.1% and 7.03 days, respectively for Bactospeine and less than 0.1% and 5.9 days for Dipel.

Over 300 isolates of *B. thuringiensis* were evaluated against the rice pests viz.: green hairy caterpillar, green semilooper, caseworm, leaf folder

and stripped stem borer (Anonymus, 1986) and it was observed that hairy caterpillar was susceptible to 150 strains, green semilooper to 93 and leaf folder to 71 and the stripped stem borer was susceptible to fewest strains.

Bell and Romine (1986) co-related the larval weight of *H. virescens & H. zea* with sub-lethal concentrations of *B. thuringiensis* and they found an inverse correlation between them.

Salama and Zaki (1986) found that the intoxication of pupae of *S.littoralis* with *B. thuringiensis* resulted in maximum malformed individuals and the moths had short life span, fecundity and fertility.

Morris (1986) tested the effect of LD_{50} dose of *B. thuringiensis* var. *kurstaki* on the weight gain by the larvae of *M.configurata* at 20 and 25^{0}C temperature. He asserted that treated 4th instar larvae registered remarkable reduction in weight gain as compared to 3rd, 5th and 6th instar larvae of the experimental insects.

Morris (1988) bioassayed Dipel 132 and Thuricide 48LV against the larvae of Bertha army worm, *Mamestra configurata* and he found both the formulations effective against the larvae of the *M. configurata*. He also reported that 4th instar larvae of *Mamestra configurata* were more susceptible in comparison to 3rd, 4th and 6th instars, when they were intoxicated with Dipel and Thuricide 48 LV. He further noted that the 3rd & 4th instar larvae elicited more susceptibility to Dipel than older instars at 20^{0}C temperature, but reverse was

true for Thuricide. He also reported that fourth instar of *M. configurata* was more susceptible to the endotoxin of San 415 than 3rd and 5th instars of *M. configurata*.

Jaquet *et.al* (1987) reported that the larvae of *Pieris brassicae* were highly susceptible to purified *B. thuringiensis* var. *morrisoni; Heliothis viresenscens* to var. *kenyae & kurstaki*, whereas *Spodoptera littoralis to entomocidus* crystal.

Joshi and Bharadwaj (1987) tested *B. thuringiensis* alone and in combination of insecticides against *S. litura*. They found that all the bacterium insecticide combinations viz.; *B.t.*+Permethrin, *B.t.* + Malathion, *B.t.*+Fenvelerate., *B.t.*+Endosulfan, *B.t.*+Quinalphos and *B.t.*+Carbaryl, resulted higher control as compared to one when used alone.

Hornby & Gardaner (1987) tested the effect of β-exotoxin of *B. thuringiensis* on holometabolous insects which elicited teratological abnormalities, reduced fecundity and longevity as a result of intoxication.

West *et.al.* (1987) tested different doses of various formulations of *B. thuringiensis var. kurstaki* against *Lambdina fiscellaria fiscellaris* by aerial application. They found Thuricide 64 B quite effective as it gave 100% suppression of the larvae when applied at a dose of 30 BIU/ha in 178 L/ha.

Jaques (1988) carried out a test in the field against the imported cabbage worm and the cabbage looper by a mixture of microbial and chemical insecticides and he observed that the protection of the crop and yield of the plots

treated with *B. thuringiensis* @0.5% and permethrin 0.1% or *B. thuringiensis* 0.5% and permaterin 0.25% were at par statistically.

Wysoki *et.al.* (1988) reported that field application of Toarow CT and Thuricide HP against *C. gnidiella*, controlled cent per cent larvae of the pest after two weeks of spray. They also assessed the bio-efficacy of Toarow CT and Dipel against giant looper, *Boarmia selenaria* at the concentraion of 0.5 and 1.0 per cent against 8 and 15 days old larvae of the test insect and reported 60-80% kill of 8 days old larvae by 0.5% concentration and 80-90% at the concentration of 0.1% Toarow CT and Dipel WP. They further stated that cent per cent mortality of 15 days old larvae occurred due to both the formulations after 8 days of treatment.

Ferre *et.al.* (1991) reported about the activation of toxins of *B. thuringiensis* which bind the receptors on the mid gut epithelium. These receptors are glycoproteins. Following binding to the epithelium, the toxins generate pores in the cell membrane disturbing cellular osmotic balance, and causing the cells to swell and lyse through a process that has been termed"colloid-osmotic lysis" (Hoftey and Whiteley, 1989).

Kimura (1991) conducted field trials to evaluate the effectiveness of formulations of *B. thuringiensis* and insect growth regulators against *Plutella xylostella* on cabbage. All applications of *B.t.* controlled the pest, but the growth regulators (chlorfluazuron and teflubenzuron) gave better control than *B.t.*

formulations (Toarow CT, Dipel, Thuricide and Bacilex).in the laboratory, LC_{50} values were 13.8 ppm. for Dipel and 6.0 ppm. for thuricide.

Artell and Richard (1992) used *B. thuringiensis* against gypsy moth, *Lymantaria dispar* and found it is effective against first and fourth instars as it effected larval-survival, growth and development time.

Thomas and Danial Hare (1993) studied the effect of different host plant consumption by *Spodoptera exigua* on *B.t.* efficacy, and reported that host induced variation and consumption rates and it is only due to the effect of *B. thuringiensis*. Larvae received lower dosage indicated other factors associated with the host plant.

Sundaram *et.al.* (1993) described the effect of spray of five chemical insecticides and three commercial formulations of *B. thuringiensis* and reported that these chemicals are effective to check the growth and development of pest in limited time.

Chio and Hau (1993) studied the effect of infections of *B. thuringiensis* to egg, larvae and pupal stage of the Asian Corn borer, *Ostrinia furnacalis* and reported that *B.t.* is pathogenic to larval stages.

Chillcott and Wigley (1993) obtained 6909 isolates *B. thuringiensis* from 455 samples of soil, insect larvae and insects habitate. Isolate tested for toxicity killed insect of order Lepidoptera, Diptera and Coleoptera. Some isolates were toxic to both Lepidoptera and Diptera and some were non toxic to any of the insect tested.

Ali and Young (1993) studied the effect of rate and total spray volume on activity of *B. thuringiensis* against *Heliothis virescens* in cotton terminals. Higher *B.t.*rates caused higher initial mortality than the lower rates and maintained higher efficacy. Persistance did not differ significantly. Spray volume did not effect the activity of *B. thuringiensis* in cotton to terminals.

Tabashink and Mc-Gaughey (1994) selected *P. interpunctella* with different *Bt.* Strains containing an array of different endotoxins and analyzed the rate of resistance development using heritability estimates. They found that resistance development in tested insect was not significant between selection with the different strains containing upto six different toxins.

Oppert *et.al.*(1994) suggested that altered protoxin activation by midgut proteinases is indeed involved in some types of insect resistance of *B. t.* Different proteases can be produced in the insect gut depending on the plant material ingested (Broadway, 1989). Such differences could influence susceptibility through slower activation or faster metabolism of the toxins.

Navon *et.al.* (1994) reported that in a screening programme of *B. thuringiensis* activity against *Lobesia botrama* ,the strain HD-263 of subsp. *Kurstaki* was selected. The LC_{50} of the strain was 21/5fg/g, more than twice that of strain HD-1. The insect was not sensitive to several aizawai strains. In field experiments in Vineyards in Israel, *B. thuringiensis* – based products applied at 100-500 g/1000m/sup/2 and with a spray volume of 100-150 litres,were more effective than organophosphate insecticides and as effective as most insect

growth regulators. Combination of *B. thuringiensis* with other chemicals did not improve insect control. The use of *B. thuringiensis* in vines with low infestation (20-30%) reduced crop damage to 0-10%.

Gareyznski and Adang (1995) studied Cry IA(c) binding in the midgut brush border membrane of *Manduca sexta* indicate that the Cry IA (c) binding protein is anchored in the membrane by a glycosylipid anchor.

Muhammad-Iqbal *et.al.* (1996) reported the efficacy of *B. thuringiensis* subsp. *Kurstaki* HD-1 (Dipel); *Bt*k Cry IA and Cry II and *Bt* subsp. Aizawai (Florbac); *Bt*a Cry IA and Cry IC was assessed against larvae from various field populations of *Plutella xylostella* (F2 generation) collected in the Cameron Highlands, Malaysia in April 1994. Evidence of resistance to *Bt*k and to a lesser extent *Bt*a is reported in these populations (LC_{50} toxicity ratios (TR) = 3-14 and 2-8 resp.), most notably in SERD2. Based on the selection experiments with SERD2, estimates of realised heritability (h2) of resistance gave very high values for teflubenzuron and *Bt*k (c.0.7) and moderate values for abamectin and *Bt*a (C.0.3). They discussed the results in relation to IPM and IRM strategies for *P.xylostella.*

West-RJ and others (1997) were applied two aqueous formulations of *B.t.* and tebufenozide (MIMIC 240 LV) aerially over commercial stands of balsam infested with *Lambdina fiscellaria*. Tebufenozide was applied once at the rate of 65.1 g a.i.in 1.86 lit./ha. The formulations of *B. thuringiensis* were applied twice at rates of 19.3 –24.1 billion international unit in 1.54-1.93 lit/ha. Nine of the

ten plots treated with the single application of tebufenozide showed reductions resulting from treatment, ranging from 3-93% within 9-11 days and 8-100% after three weeks. Plots treated with *B. thuringiensis* had larval population reductions from 76 to 93 per cent 10 days after first application and from 98 to 100% 7 days after the second application. No pupae were recovered from such plots.

Saxena (1998) studied the toxicity of thuricide HP against gypsy moth, *Lymantria dispar.* He used this insecticide by spraying method. He observed that the percentage of infested plants decrease in ten days after treatment. Hence, the productivity increased in short period. Larval survival and development period is also increased.

Trisyono and Whalon (1999) studied the toxicity of Neem and combination of neem and *B. thuringiensis* on 2nd instar of the *B. thuringiensis* susceptible and resistant strains of colorado potato beetle, *Leptinotarsa decemlineata* (Say) using a potato leaf dipping method, the LC_{50} values in both strains decreased significantly with increased exposure time- sub lethal concentrations of neem or *B.t.* applied separately or in combination decreased the larval weight and retarded the larval growth. The performance of neem in combination with *B.t.* on field population of colorado potato beetle were warranted.

Chandra *et.al.* (1999) studied the effectiveness of *B. thuringiensis* (*B.t*) based products, Biolep; Dipel, and Biobit (*Bt* .sub sp. *Kurstaki*) was tested against the third instar larvae of *Helicoverpa armigera* in environmentally

controlled conditions. The LC_{50} of *Bt.* products, Biobit, Biolep and Dipel for third instar larvae of the pest insect were 0.114, 0.211 and 0.213 per cent respectively for the exposed period of 48 hours and for the post exposure period (till pupation) the LC_{50} were 0.087, 0.186 and 0.159 per-cent. All the concentrations of *B.t.* products have adverse effect on growth and development of the test insect, increased larval mortality, larval period, growth inhibition and decreased pupation, pupal weight and adult emergence was recorded with increased dose of *B.t.* under laboratory conditions dose.

Earworm larvae were exposed to artificial diet treated with increasing *B.t.* concentrations, and mortality and growth inhibition were evaluated after 7 days. The range of variation in *B.t.* susceptibility indicated by growth inhibition was very similar to that indicated by mortality (Siegfried *et.al.*, 2000).

Navrozidis *et.al.* (2000) used *B. thuringiensis*, isolate 114A in toxicity experiments against the wild population of the olive pest, *Bactrocera oleae.* In laboratory experiments, spores and crystals of the *B.t.* were delivered to the insects with the food. Longevity, oviposition period, number of eggs produced and per cent hatch were recorded. It was found that, in addition to the longevity, the oviposition period, number of eggs and per cent egg hatch decreased. Also, the percentage of pupation and emergence was reduced when olive fruits with eggs in their mesocarp were dipped in the solution of spores and crystals. Field applications with the toxins of 114A isolate of *B.t.* have resulted in significant protection of the olive production.

Nault *et.al.* (2000) examined the feeding, development and survival to adulthood of colorado potato beetle after exposing large larvae to *B. thuringiensis*. They observed that the third instar remaining on plants after a *B.t.* application were unlikely to feed and 4th instars consumed only 50 per cent as much foliage as those fed untreated leaves. Many late instars subjected to *B.t.* treated foliage failed to survive to adult hood, 58-83% of these beetles died during larval stage. Reduced feeding and poor survival of late instars suggest that counts of large larvae after application do not provide a complete picture of the efficacy of the *B.t.* treatment. Due to treatment of foliage the larval period extended upto 4.5 days. Delayed emergence of adults that feed on *B.t.* – treated potatoes as late instars indicated that development was prolonged in these insects because of ingestion of a sub-lethal dose of *B. thuringiensis.*

Bulbulshoev and Bulbulshoeva (2000) investigated new local pathogenic strains of *Bacillus thuringiensis* to control insect pest of woody plants. Twelve strains were highly virulent on caterpillars of cabbage large butterfly and yellow moth, while five strains were virulent on apple moth. Control of the brown tail moth did not exceed 47 per cent.

Sharma *et al.* (2001) evaluated the toxicity of *B.t.* vars. *kurstaki* and *aizawai* against some lepidopterous larvae by leaf/ fruit dip method. Larvae of the test insect were released on the treated food. Mortality of the test insect was recorded 1,3,5 and 7 days after their release. The larvae of *Antigastra catalaunalis* were observed to be the most susceptible to both the varities of *B.t.* showing 100 % mortality one day after release.

The combined action of *Pongamia pinnata with Bacillus thuringiensis* var. *kurstaki* (*B.t.k.)* at 25, 50, 100 150, 200 and 300 ppm on mature larvae of *A. diaperinus* was investigated by Hasan *et al.* (2002). Results showed that the efficacy of (*B.t.k.) was significant the* LD_{50} value was 131.372. The pupation also decreased significantly ($P < 0.001$, $F = 136.37$). The adults emergence also decreased significantly ($P< 0.001$, $F = 70.677$). There was no effects on sex ratio.

Chaturvedi (2003) studied the effectiveness of *Bacillus thuringiensis* Ber. against *Utetheisa pulchella* L. and reported that Dipel, Thuricide and Bactospeine proved best controlling agents without disturbing our ecosystem. They effect growth and development of pest and caused remarkable .mortality in larvae. *B.t.* preparations also caused prolongation in larval and pupal period. Loss of weight in larvae and pupae was also observed.

Fetoh and Azazy (2004) conducted laboratory and field expereiments to evaluate three biological control agents for the control of the cabbage worm as new approaches of IPM. Under laboratory conditions, larvae and pupae were highly susceptible to dipel. Dipel exhibited highly toxic effect against larvae (93.3% mortality). Under field conditions, the mortality 7 days after applications was 76.1.

Devaki and Krishnayya (2004) conducted an experiment to evaluate the effects of the combination of *B.t.* formulations (Dipel 8L, Delfin WG and Halt WP) and neem products against third instar larvae of *Spodoptera litura.*

Workers observed that the percentage of larval mortality was highest when *B.t.* formulations were combined with neem (84.5 to 89.5%). The percentage of feeding inhibition due to the *B.t.* formulations and neem products ranged between 30.6 to 32.4. The solo treatments of *B.t.* formulations, neem products and their combinations showed significant effect on the pupation and adult emergence of *S. litura.*

Baker (2004) conducted a field study to determine the effects of the additions of sugar as stimulant in improving the efficacy of *B.t.* based product (Dipel – 2x) against larvae of grape moth. The addition of sugar as feeding stimulant to a 50% reduced field rate of Dipel – ex resulted in higher control rates (80%) against the larvae compared to using the recommended field rates of Dipel – 2x alone.

Naglaa *et al.*, (2004) used commercial formulations of *B.t.* to control the larvae of greater wax moth, *Galleria mellonella* and reported Lc_{50} was 4.784 g/100 g diet. The Lc100 (9.568 g/100g diet or water) was used to treat the wax combs and foundation wax sheets by spraying. Infestation was completely reduced in Dipel – treated wax combs and foundation sheets.

Kumar and Gujar (2005) determined the toxicity of *B.t.* Cry IA delta – endotoxins to the six day old larvae of diamond back moth by leaf dip method. Workers found Cry IAb was two times more toxic than Cry Dr. I.C. The median lethal concentrations, Lc_{50} 72h, varied from 0.002 to 0.386 micro g/ml for Cry IAb and from 0.011 to 0.324 micro g/ml for Cry IAc. The winter population of insect

showed significantly lower (4 – fold) susceptibility to Cry IAb than the summer population at Hisar.

Gopalakrishnan and Gangavisalakshy (2005) tested efficacy of Deflin, Halt, Dispel DF and Biobit against *Papilio demoleus* and reported that 5 applications of *B.t.* formulations at 1kg/ha effectively controlled the larval population of *P. demoleus* compared with the untreated control.

Vastrad *et al.*(2005) used endosulfan, monocrotophos, methomyl, fenvelerate, cartap and *B. thuringiensis* to control diamond back moth by using leaf dip, larval dip methods and found leaf dip method was ideal for monitoring insecticide resistance but slopes resulting from the different bioassay methods did not differ significantly.

Li *et. al.*, (2005) evaluated Dipel resistant and susceptible strains of *Ostrinia nubilalis* for larval mortality and growth inhibition when fed diets containing individual *B.t.*protoxins. Resistance ratios for four of the protoxins in Dipel (Cry IAa, Cry IAb, Cry ICc and 2Aa) were 170, 205, 524 and 640 fold respectively, considerably higher than the 47 – fold resistance to Dipel. The Dipel –resistant strain was 36 – fold resistant to Cry IBa, a protoxin not present in dipel. Cry ICa did not cause significant mortality for resistant/ susceptible larave.

Hernadez *et al.*,(2005) isolated *B.t.* from 116 samples collected in high altitude potato growing areas. *B.t* .was found in 60% of the samples. The main percentage of samples with *B.t.* was found in larvae of *R. tucumanus* (78%). Bioassays were performed with 112 isolates. None resulted toxic to either

larvae or adults of weevils. They reported most toxic strains against *Spodoptera exigua* were Cry gene content.

CHAPTER THREE

CHAPTER - THREE

MATERIALS AND METHODS:

In the present chapter details of materials used and then techniques employed for different experiments of this investigation are dealt here with under following heads-

INSECT:

3.1A. Source:

Male and female *Diacrisia obliqua Walker* (Lepidoptera: Arctiidae) were collected in the second week of August, 2003 and the same were brought to the laboratory for maintaining their stock for different experiments. The larvae and adults employed in laboratory studies were obtained from this stock which was maintained throughout the tenure of the present investigation.

3.1B. Marks of Identification:

Moths are medium sized with a wing expanse of about 50 to 66 mm. The wings are pale buff coloured. Abdomen of moth is crimson with black spots. Males and females are usually identical in appearance. The female is bigger than male. The abdomen of female is wider and stumpy. Adults mate soon after emergence.

3.1C. Host plants and damage:

D. obliqua is a polyphagous insect. Moths feed on sesamum, linseed, safflower, castor, jute sunflower, potato, tomato, several seeds and

cause mild to severe loss. The hairy caterpillars feed on leaves, buds and flowers of different host plants. In case of severe infestation, the plants may be completely defoliated.

3.1D. Life Cycle of *Diacrisia obliqua Walker:*

A female lays about 280-450 eggs during her life period. The eggs are deposited in clusters. The eggs are small, spherical and pale or yellow in colour and then gradually turn deep yellow. The egg stage lasts for 2-4 days. The newly hatched larva is dark grey with yellow bands on the body, measuring about 2 mm. in length. The full grown larva measuring 28 to 40 mm in length is hairy orange with head black and the last two segments black. The caterpillar march from field to field in large numbers causing destruction. It moults four times to make the number of instars five. The larval period lasts for 13 to 28 days. The larva, having attained the full growth, pupates in soil or on the ground in a silken cocoon. The pupa is reddish brown measuring upto 2 cm in length. Adult emerges out form the pupa in 4 to 10 days, depending on the season. The life cycle is completed in 26 to 45 days.

3.2 LABORATORY STOCK OF THE MOTH:

A stock of the moth was maintained in the laboratory to ensure its regular supply of different developmental stages in a large number for different studies during the investigation. For this purpose, the moth was reared in large number, generation after generation.

Moths, males and females obtained from field & were maintained in glass chimneys provided with twenty per cent sugar solution with *Crotalaria* leaves for oviposition. Eggs obtained from them were kept as such for hatching.

On their hatching, larvae obtained were reared on tender *Crotalaria* leaves, in large petridish to grow in groups of twenty per petridish upto second instar stage. The food supply was maintained twice a day in view of evaporation of water from leaves. Precaution was taken to avoid infection or contamination. After the second instar stage the larvae were reared in groups of five in petridishes. For these larvae also, the food supply was maintained as described above till the pupation. When larvae acquired full growth and stopped feeding, they were transferred in pneumatic troughs having about 10 to 15 cm thick soil on their bottoms. The larvae pupated either on the surface of the soil or little below the surface of the soil. Pupae, thus obtained were kept for emergence of moths. On emergence, adults were maintained as already described above for obtaining eggs. From larvae of these moths, next generation was reared as described above and in the same way the insect was reared generation after generation and the continuous supply of this moth throughout the whole tenure of the investigation was ensured.

3.3 BIOCHEMICALS USED:

The following commercial preparations of *B. thuringiensis* whose efficacy as controlling agents has already been evaluated among different

insects by different economic entomologists were employed against *Diacrisia obliqua* in this study.

Name of commercial preparations of *B. thuringiensis* used in this investigation are mentioned below-

1. Dipel wettable powder containing $25x10^9$ viable spores per gram of final product of *B. thuringiensis* var. *kurstaki* (Serotype 3 a, b strain HD-1).
2. Thuricide H.P. wettable powder containing $30x10^6$ viable spores of *B. thuringiensis* var *kurstaki* (serotype 3a, b strain HD-1) per gram of final product.
3. Bactospeine containing $1x10^8$ viable spores of *B. thuringiensis* (serotype-1) per gram of final product.

3.4. CONCENTRATIONS OF BIO CHEMICALS USED:

The commercial preparation of *B. thuringiensis* namely Dipel, Thuricide HP and Bactospeine were obtained in pure form, and stock solution used in the present investigation was prepared in distilled water. One gram of Dipel, Thuricide HP and Bactospeine were mixed separately in 100 ml of distilled water which gave the concentrations of one per cent, from this, other concentrations were prepared by serial dilution method.

Two per cent skimmed milk powder was added to bacterial suspension for improving the adhering quality of the bio-chemical. The

concentrations were applied against *D. obliqua* in the studies included 0.05, 0.10, 0.50, 0.75 and 1.0 per cent.

3.5. METHODS OF APPLICATION OF Bt. INSECTICIDES :

The experimental insects were treated with different concentrations of bacterial preparations by following two methods.

3.5A. Leaf dip method:

In this method of treatment small and uniform size of leaves of host plant were treated with each concentration of particular bacterial preparation by leaf dip method.

3.5B. Topical method:

In this method of treatment about 2 hr old adults were exposed to a thin film of residue of a concentration of a particular bacterial preparation. For obtaining the thin film of bacterial preparation as residue, about 10 ml of a concentration of bacterial preparation was poured in a petridish (10 cm dia.) and the petridish was tilted in different ways to spread the bacterial preparation on the whole floor area of the petridish and its raised periphery. This led to the formation of a thin film of a concentration of bacterial preparation in the petridish as residue. Adults were left in petridishes having thin film of the bacterial preparation for 24 hours. The petridishes were covered by thin muslin cloth to prevent the escape of the adults. Such treated adults were employed in the different experiments as described later on.

3.5C. Control Experiment:

Control experiments were also set up to evaluate and compare the findings of different experiments. In control feeding experiment the same procedure as of feeding treatment was adopted but the *Crotalaria* leaves that were given to the larvae were not treated in the bacterial solution. They were simply dipped in two per cent skimmed milk solution. In the topical control experiment only 2 per cent skimmed milk solution was poured in the petridish. Rest of the operations and observations were done in similar manner for different aspects, as taken in the feeding and topical treatments.

3.6. DESIGNES OF STUDIES:

Studies presented in this thesis were conducted experimentally under laboratory conditions of temperature and relative humidity. These studies were carried on under following headings:

A. The effect of bacterial preparations on growth.

B. The effect of bacterial preparations on development.

C. The effect of bacterial preparations on fecundity and fertility.

D. Sex specific sterility effect of bacterial preparations on sexes.

E. Study of the compatibility of *B.thuringiensis* with chemical insecticides.

The above mentioned aspects were studied as under:

3.6A. Effect of bacterial preparations on growth:

This aspect was studied in terms of accumulation of biomass in larva at regular intervals and acquisition of biomass in both pupa and adult and was evaluated as under-

3.6 A.1. The influence of bacterial preparations in larva pupa and adult:

This was studied under two different conditions of treatment (leaf dip method and topical method). In both conditions, the adult insects were treated with a strength of bacterial preparation by leaf dip method and topical method. The influence of a bacterial preparation on biomass accumulation in larva under both treatments was studied as follows.

3.6A.1a. The influence of bacterial preparations on biomass accumulation in larva under treatment by leaf dip method.

This was studied by employing larvae on the leaf treated with different concentrations. The influence of a bacterial preparation on the larval growth under this treatment was studied by five experiments, one for each strength, each consisting of three replicates. Twenty larvae (1/2-1 hr old) per replicate were reared on tender leaves of *C.juncea* till the 16^{th} days of development. The weight of these larvae was recorded on the 5^{th}, 10^{th} and 15^{th} day of their larval duration. These records were obtained with reference to each strength of each bacterial preparation. The experiment designed to determine the influence of a bacterial preparation were accompanied by a control experiment.

3.6.A.1b. The influence of bacterial preparations on biomass accumulation in larva under treatment by topical method:

The larvae obtained from the adult treated with different strengths of the bacterial preparations by the topical method were employed for evaluation of their growth. The influence of the bacterial preparations on the growth was determined with reference to identical five strengths of each bacterial preparation exactly on the above mentioned pattern and the related records were obtained with reference to them.

The experiments for a bacterial preparation were accompanied by a control experiment also.

3.6.A.II. Effect of bacterial preparations on weight acquisition by pupae and adults:

This aspect was studied by applying the bacterial preparations by leaf dip method and topical method to adults. The adults were treated by topical method while larvae by leaf dip method.

3.6A.IIa. Effect of bacterial preparations on weight acquisition by pupae & adults under leaf dip treatment:

Sixty larvae were selected at random from the laboratory stock and were treated with a strength of bacterial preparation by leaf dip method. These treated larvae were maintained in glass chimneys for the development. After that one glass chimney housed one pair of adult. When oviposition occurred, eggs obtained were kept on moist filter paper for their hatching. Sixty larvae of such

eggs were selected at random and divided into three groups of twenty larvae, each group constituted a replicate. The larvae of the replicate were reared on tender leaves of host plant until they pupated. The pupae thus obtained when acquired 4 to 6 hr age, were weighed and their weight was recorded. These pupae were kept for emergence of adults. On emergence and after having the discharge the meconium the adults were weighed after one hour and their weight was recorded. The aforesaid study was conducted with reference to each strength of every bacterial preparation tested and records identical to above mentioned were obtained.

3.6.AII.b. Effect of bacterial preparations on weight acquisition by pupae and adults under topical treatment:

Twenty pairs of adults selected at random from the rearing stock and were treated with a strength of a bacterial preparation for 24 hr in petridishes and thereafter, they were maintained in glass chimneys for oviposition. The eggs deposited were kept for hatching and the sixty larvae of such eggs were selected indiscriminately. These larvae made three replicate and were reared to obtain their pupae and adults. The pupae when 4 to 6 hr old were weighed and their weight was recorded. The adults were also weighed, about one hr old, after discharge of meconium and subsequently their weight was recorded.

3.6B. EFFECT OF BACTERIAL PREPARATIONS ON DEVELOPMENT:

The effect of different strengths of all the considered bacterial preparations on development of *Diacrisia obliqua* was studied in response to their application to moth by leaf dip method and topical method as described below:

3.6B.1 Effects of bacterial preparations on development under leaf dip method of treatment:

This was studied experimentally with immediately hatched larvae obtained from females fed on a strength of a bacterial preparation were employed experimentally. This was tested by one experiment designed separately for each strength of bacterial preparation. Twenty such larvae per replicate were reared on tender leaves of host plants until their pupation, number of larvae pupated, their developmental duration and survival were recorded. These pupae were kept to obtain adults. On emergence of adults, the number of adults emerged and their pupal period were recorded besides these, the sex ratio of adults was also recorded. The experiment was further extended to record the life duration of males and females. For this purpose males and females were maintained individually date wise in glass chimneys on daily supply of twenty per cent sugar solution till their natural death and on their expiry, their longevity was recorded. Besides, for the purpose of comparison a control experiment was also set for each strength of bacterial preparation.

3.6B.II. Effect of bacterial preparations on development under topical method of treatment:

This was studied experimentally with newly hatched larvae of adults which were already forced to contact their residue film of a strength of each bacterial preparation. It was determined in one experiment which consisted of three replicates. Twenty such larvae were reared on leaves of *C.juncea* till their pupation and when they pupated, the number of pupae obtained and the larval period were recorded. The pupae were kept in glass chimneys date wise and when moths emerged from them, their number and pupal period were noted. Besides, this the sex ratio was also recorded. The experiment was further extended for recording the life span of male and female moths. For this purpose males and females were maintained individually date wise in glass chimneys on daily supply of twenty per cent sugar solution till their natural death and on their expiry, their longevity was recorded.

Besides the above records under different methods of application of bacterial preparations, the record pertaining to net mortality was obtained as suggested by Abbot (1925) as follows:

$$\%\,\text{Net mortality} = \frac{\%\ \text{Mortality in test} - \%\,\text{Mortality in normal}}{100 - \%\,\text{Mortality in normal}} \times 100$$

3.6C. Effect of bacterial preparations on reproduction:

The reproduction in *D. obliqua* under influence of different bacterial preparations was studied under two headings —

1. Effect of bacterial preparations on reproductive periods and fecundity.
2. Effect of bacterial preparations on fertility and incubation period.

3.6.C.1. Effect of bacterial preparations on reproductive periods and fecundity:

The pre-oviposition and oviposition periods and the number of eggs laid by a female were studied separately by applying bacterial preparations to larvae and adults as described under.

3.6C.1a. Effect of bacterial preparations on reproductive periods and fecundity under leaf dip method of treatment:

Ten males and ten females were obtained indiscriminately from the earlier treated stock. The females were maintained individually with a male in glass chimney on daily supply of twenty per cent sugar solutions, for oviposition. When these females laid eggs for the first time, pre-oviposition period was recorded. The females were maintained till they laid last egg and after that their oviposition period were recorded and their total number of eggs was counted. The above study was made separately for each strength of all the tested bacterial preparations and above mentioned records were obtained for them. A control experiment was also set for each bacterial preparation.

3.6C. 1b. Effect of bacterial preparations on reproductive periods and fecundity under topical method of treatment:

Ten females along-with ten males were selected at random from the laboratory stock. Both males and females were compelled to contact a thin film of strength of bacterial preparation for 24 hrs. Thereafter, each female was maintained in a glass chimney with a male on twenty per cent sugar solution. They were kept as such for egg laying and when the first egg laid, the pre-oviposition period was recorded. The females were maintained till the deposition of their last egg, after which the oviposition period was recorded. The total number of eggs laid during the oviposition period was recorded. The above mentioned study was conducted separately for all concentrations of the tested bacterial preparation and the above mentioned records were obtained for them. Besides, a control experiment was also designed for each bacterial preparation.

3.6c.II. Effect of bacterial preparations on fertility and incubation period:

The influence of bacterial preparations on fertility and incubation period in *D. obliqua* was studied with reference to leaf dip method and topical method as follows:

3.6C.IIa. Effect of bacterial preparations on fertility and incubation period of *Diacrisia obliqua* under leaf dip method of treatment:

Ten females and ten males, each 1 hr old were selected indiscriminately from the earlier treated laboratory stock. These moths were maintained as pairs in glass chimneys with twenty per cent sugar solution. Each pair constituted a replicate. The eggs from each replicate collected daily and kept date wise on moist filter paper. On hatching of the eggs, the number of eggs hatched and their incubation period were recorded. The above study was undertaken with reference to different strengths of all the tested bacterial preparations and the above mentioned records were obtained. A control experiment was set for every bacterial preparation and similar records were maintained.

3.6C.IIb. Effect of bacterial preparations on fertility and incubation period of *Diacrisia obliqua* under topical method of treatment:

For the study, ten males and ten females were drawn indiscriminately from the laboratory stock. These moths were compelled to contact thin residue film of a strength of bacterial preparations for 24 hrs and thereafter these were maintained as pairs in glass chimneys with twenty per cent sugar solution; each chimney had one pair of moth. Each moth pair made a

replicate. Eggs from each replicate were collected daily and kept date wise on moist filter paper. On hatching of the eggs, their viability and incubation period were recorded. The experiment for each bacterial preparatoin was accompanied by a control experiment.

Besides the above mentioned records, the records pertaining to the reduction in the fecundity, net sterility and control over reproduction were also obtained as described below:

The reduction in the fecundity was calculated following the formula of Chamberlain (1962) as detailed below:

$$\%\,\text{Reduction in fecundity} = \frac{\text{Eggs laid in normal} - \text{Eggs laid in test}}{\text{Eggs laid in normal}} \times 100$$

The sterility was calculated following the formula of Abbot (1925) as detailed below:

$$\%\,\text{Net sterility} = \frac{\%\,\text{Sterility in test} - \%\,\text{Sterility in normal}}{100 - \%\,\text{Sterility in normal}} \times 100$$

The control over the reproduction was calculated by following the formula of Chamberlain (1962) as detailed below:

$$\%\text{Control over reproduction} = \frac{\text{Eggs hatched in normal - Eggs hatched in test}}{\text{Eggs hatched in normal}} \times 100$$

3.6.D. Sterility effect of bacterial preparations on sexes:

Studies described at serial 3.6A did not project the sex specific influence of the tested bacterial preparations. In order to determine this, the following study was carried by monitoring matings between treated female and

untreated male and between untreated female and treated male. This was studied with reference to each strength of all the tested bacterial preperations separately by two experiments, each consisting of three replicates. All experiments were set as described in 3.6C. Experiments were also accompanied by control experiment.

3.7. Compatibility of Insecticides with Dipel:

On the basis of screening different commercial formulations of *B. thuringiensis*, Dipel was found most effective. Therefore, compatibility of insecticides was studied with Dipel only.

To study the compatibility of insecticides with Dipel against the larvae of *D. obliqua,* the two experiments were set. First experiment comprises the evaluation of insecticides while the second experiment was set for the evaluation of insecticides of combination with the pathogen. Six commercial insecticides namely; Endosulfan, BHC, Quinalphos, malathion, Fenvelerate and Cypermathrin were tested for their relative toxicity by the following technique.

Five concentrations of each insecticide were taken and the leaf dip method as described earlier was adopted for the leaf treatment. For determining the relative toxicity of insecticides, small, uniform leaves of castor were treated with different concentrations of insecticides and five days old larvae, starved for twelve hours, were released for twenty four hours. Thereafter, fresh untreated leaves were provided to them for feeding. The larval mortality was recorded after

24, 48, 72, 96 and 120 hours of treatment. The data, thus obtained after 120 hours, were utilized for the assessment of relative toxicity of insecticides tested.

Similarly, in the second experiment, a constant sub-lethal concentration of Dipel (0.05%) was mixed to each concentration of insecticide tested and bioassay was made against the test larvae as described earlier. The data noted on larval mortality were subjected to statistical analysis for working out the LC_{50} value of each insecticide in combination with Dipel.

The toxicity index was also worked out for insecticides and their combination with Dipel by taking the LC_{50} of Malathion as unit. The toxicity index was calculated with the help of following formula;

$$\text{Toxicity index} = \frac{Lc_{50} \text{ of insecticide A}}{Lc_{50} \text{ of insecticide B}}$$

Where A=Lc_{50} of Malathion

B = Lc_{50} of other tested insecticide

With each set of the experiment a control experiment was also set, where the larvae were treated with distilled water.

3.8. STATISTICAL ANALYSIS:

Various statistical analysis mentioned below have been applied to study the nature and relationship between variables, to know the reliability and precision in the results obtained, to test the significant difference between the observed and corresponding expected values and to predict the estimated values of effectiveness for a given value of concentration.

3.8a. Standard error:

Standard error is used for estimating the errors which are likely to be there in the average of the values obtained in the difference replicates of experimental treatments. The standard error has been calculated with the help of the following formula.

$$SE=\frac{SD}{\sqrt{n}}$$

Where,

n = Number of observations.

S.D.= Standard deviation.

Standard deviation was calculated by the following expression.

$$S.D.=\sqrt{\sum\frac{(X-x)^2}{n}}$$

Where,

x = observation of variate values.

X = Arithmetic mean of the observation.

n = Number of observations.

3.8b. Significance test:

The significance test was done to reveal, whether the differences in the results obtained at the different levels were due to errors of sampling or there existed real differences between the treatments.

(a) Chi Square test (x^2test):

For testing the independence or association between the effectiveness and concentrations, x^2- test was also used. The heterogeneity of the data were tested maximum at 5 per cent probability level.

3.8.c. Regression equation:

Regression is the measure of the average relationship between two or more variables in terms of the original units of the data. Regression lines between probit mortality/reduction in larval growth and log concentration was determined to predict the estimated value of effectiveness at the given value of log concentration. (Finney 1952).

3.8.d. Determination of E.D. 50:

Different $E.D._{50}$ values were calculated with the help of the regression equation to know the concentration/dose causing, 50% mortality/reduction in food consumption/reduction in larval growth.

3.8.e. Relative efficacy:

Relative efficacy was calculated by dividing the different values by suitable standard value.

CHAPTER FOUR

CHAPTER - FOUR

RESULTS AND DISCUSSION:

Diacrisia obliqua Walker is a phytophagous insect causing great loss to different crops. The administration of different microbial preparations (Dipel, Thuricide, Bactospeine) was done by leaf dip method and topical method to *Diacrisia obliqua* to evaluate the effect of these biological preparations on the growth and development of target insect. These effects were studied under different headings such as effect on growth, effects on post embryonic development, effect on reproduction and their sterilizing effects on male and female *Diacrisia obliqua*. Toxicity of different concentrations of endosulfan, B.H.C., malathion, Quinalphos etc. against five days old larvae of *D. obliqua* also studied. Efficacy of Dipel in combination with chemical insecticides against the five days old larvae of *D. obliqua* also studied. The results obtained in different experiments are presented in following pages. Different tables and graphs are also presented in suitable places of this chapter.

4.1. EFFECT OF BACTERIAL PREPARATIONS ON GROWTH:

4.1.A. Effect of dipel on growth:

4.1.A.a. Effect of dipel on biomass accumulation in larva under leaf dip method:

Larva of the control experiment accumulated 4.28 mg biomass on the 5th day of its life. Whereas the larval biomass on the same day varied from 1.88 to 3.82 mg under influence of different concentrations of dipel. The control larva acquired significantly more biomass than that of the larva under influence of any strength of the dipel used ($P<0.01$). The larva under the effect of 0.05 per cent dipel had more weight (3.82 mg) than that obtained under the influence of 1.0 per cent concentration of dipel (1.88 mg ; $P<0.05$). Further, analysis of variance revealed that 0.05 and 0.10 concentrations had almost similar effect on the biomass accumulation (3.26 to 3.82 mg) on the 5th day of the larval period. But at any of these concentrations the larva had more biomass than that of the larva under the influence of 0.50, 0.75 and 1.00 per cent (2.89 mg, 2.26 mg and 1.88 mg) concentrations ($P<0.05$). Thus, on the basis of the biomass accumulation in the larva on 5th day, the tested concentrations of dipel could be arranged as 0.05%>0.10%>0.50%>0.75%>1.00% (Table -1).

On the 10th day of its life, the control larva had 22.68 mg biomass which was significantly more than that of the larva on the same day under the influence of any strength of dipel from 0.05 to 1.00 per cent ($P<0.01$). In response to treatment of different concentrations of dipel, the weight of larva varied form 6.66 to 18.38 mg and analysis of variance test revealed that the biomass of the larva on this day differed significantly with the strength of the dipel

(P<0.05). The biomass of the larva showed the tendency of decrease with increase in concentration of the dipel on the 10th day of the larval period (Fig. 1).

The biomass of the control larva was 98.86 mg on the 15th day and it was significantly more than that of the larva on the same day under influence of any concentration of the dipel used (P<0.01). In response to treatment with different concentrations of dipel, the biomass of the larva on the 15th day varied from 23.26 to 85.46 mg and it differed significantly with the strength of the dipel (P<0.01). The biomass in larva decreased with increase in the concentration of the dipel. The tested concentrations of dipel could be arranged as 0.05%>0.10%> 0.50%> 0.75%> 1.00% (Table -1; Fig. -1).

4.1A.b.Effect of dipel on biomass accumulation in larva under topical method :

Larva of untreated adult acquired significantly more weight (4.28 mg) on the 5th day in comparison to larva of treated adult with any concentration of dipel (P<0.05). Further, the biomass of the larva varied from 1.94 to 3.96 mg in response to treatment of different strengths of dipel under topical method and it appeared to decrease with increasing concentrations of the dipel. At 0.05 per cent concentration of the dipel, larva had more weight (3.96 mg) than that acquired by it (1.94 to 3.66 mg) at any of the other concentration of dipel (P<0.01). Weights of larva at 0.05 and 0.10 per cent concentrations (3.96 and 3.69 mg) were not different statistically (P>0.05) but biomass of larva at any of

these concentrations was certainly more than that acquired at 0.50, 0.75 and 1.00 per cent concentration of the dipel (P<.0.05) (Table – 1).

On the 10th day also, the larva of the adult not treated earlier with dipel acquired more weight (22.68 mg) than that which was treated earlier at adult stage with any concentration of dipel (P<0.05). In response to treatment with different concentrations from 0.05 to 1.00 per cent, the larval biomass varied from 6.84 to 17.78 mg and it differed with the concentration (P<0.05) and decreased with increasing concentraion of this bacterial controlling agent.

The control larva on 15th day accumulated 99.90 mg biomass, whereas it obtained 24.85 to 86.49 mg in response to treatment earlier at adult stage with different concentrations of dipel from 0.05 to 1.00 per cent and it differed with the concentration (P<0.01), tending to decrease with increasing concentrations.

Corresponding concentrations under both methods of treatment exerted similar influence on the larval biomass on 5th, 10th and 15th day. The larval biomass under topical method was more (1.94 mg) than acquired by larva treated with leaf dip method (1.88 mg) on 5th day. On 10th and 15th day of treatment, the larva treated under topical method also acquired more weight (6.84 mg and 24.85 mg) than acquired by larva (6.66 mg and 23.26 mg) under leaf dip method. So it was found that any concentration of dipel was found more effective on 10th and 15th day under leaf dip method (Table – 1).

Table - 1

Effect of different concentrations of "Dipel" under different modes of treatment on biomass accumulation in larva of *D. obliqua*.
(Values are mean ± S.E.)

Mode of treatment	Concentration (%)	Larval biomass (mg) ± S.E. on		
		5th day	10th day	15th day
L.D.M.	0.05	3.92 ±0.13	18.38±0.34	85.46±0.58
	0.10	3.26±0.15	15.88±0.25	78.04±0.43
	0.50	2.89±0.16	13.07±0.26	49.48±0.48
	0.75	2.26±0.16	9.08±0.18	38.33±0.34
	1.00	1.88±0.10	6.66±0.26	23.26±0.68
T.M.	0.05	3.96±0.10	17.78±0.24	86.49±0.30
	0.10	3.69±0.12	15.68±0.34	79.37±0.24
	0.50	3.06±0.14	13.24±0.14	58.48±0.34
	0.75	2.86±0.16	9.63±0.18	38.86±0.58
	1.00	1.94±0.15	6.84±0.24	24.85±0.77
	Control	4.28±0.16	22.68± 0.52	99.90±0.62

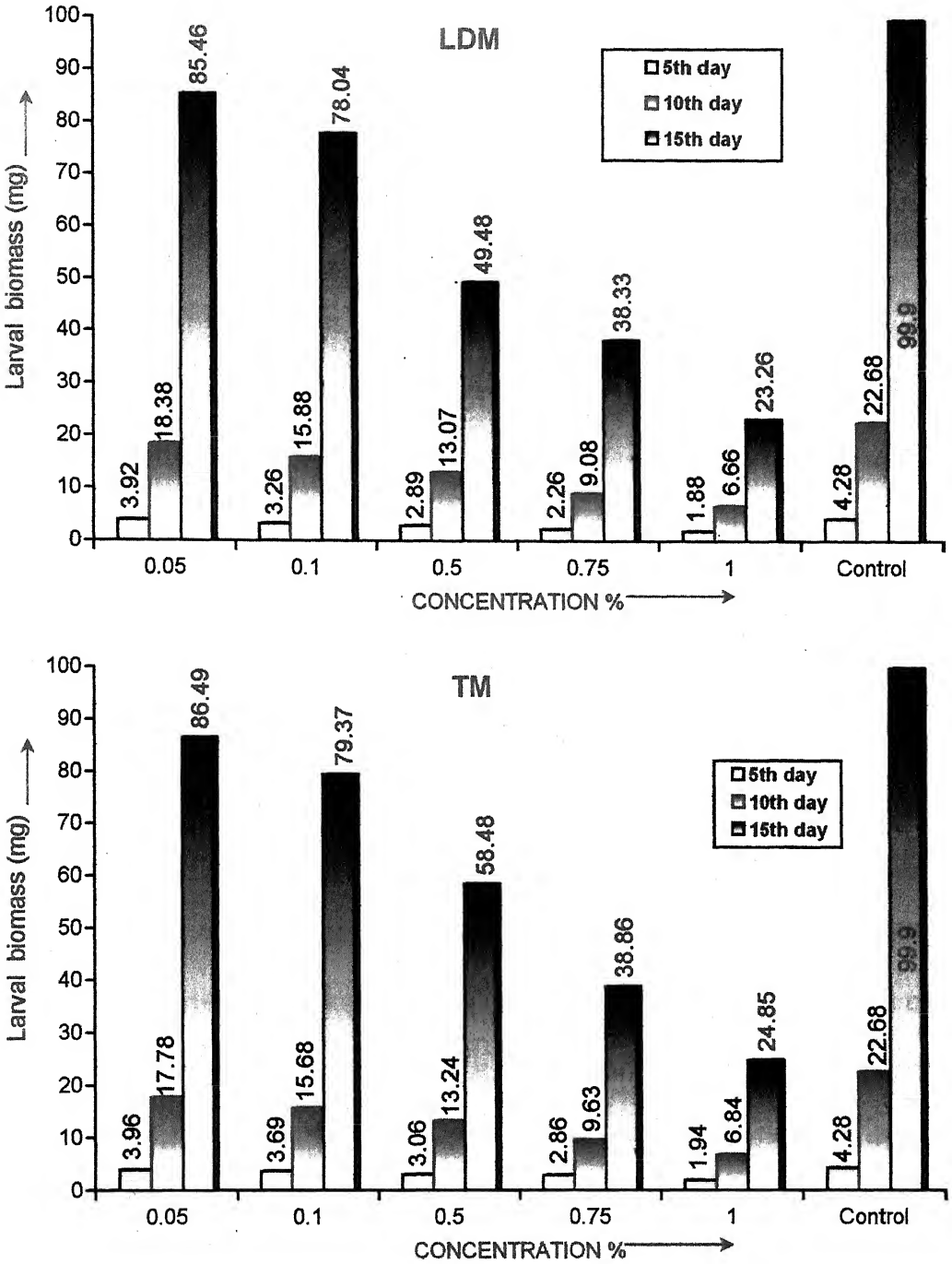


Figure 1. Effect of different concentrations of "Dipel" under different modes of treatment on biomass accumulation in larva of *D. obliqua* on 5th, 10th and 15th day.

4.1A.II. Effect of dipel on biomass acquisition in pupae and adults:

4.1A.IIa.Effect of dipel on biomass acquisition in pupae and adults under leaf dip mehtod:

Pupa obtained from the male and female treated with any of the concentrations of the dipel was considerably lighter than that obtained from the untreated moths ($P<0.01$). The biomass of the pupa in response to treatment with different concentrations of the dipel varied from 64.46 to 128.46 mg, decreasing with the increasing concentrations and it was detected to depend on the concentration (Anova, $P<0.01$)(Table -2).

Like the pupa, the male moth obtained from the untreated pupa was heavier (99.87 mg) than that obtained from the pupae treated with any concentration of the dipel. Weights of the male moth varied from 46.63 to 89.48 mg in response to the pupal treatment with different concentrations of the dipel and as per analysis of variance, the weight of the male moth depended on the concentration of the dipel ($P<0.01$) with a clear tendency of decrease with increasing concentration (Table – 2).

The untreated parents adult female acquired significantly more biomass (103.65 mg) as compared to that obtained from parents treated with any concentration of dipel ($P<0.01$). In response to treatment with different concentrations, the weight of the female varied from 53.62 to 95.66 mg tending to

Table - 2

Effect of "Dipel" at different concentrations under different modes of treatment on biomass accumulation by pupa and adults of *D. obliqua*.
(Values are mean ± S.E.)

Mode of treatment	Concentration (%)	Weight (mg) ± S.E.of		
		Pupa	Male	Female
L.D.M.	0.05	130. 46±0.36	89.48±0.38	95.66±0.48
	0.10	121.76±0.47	78.38±0.36	88.52±0.42
	0.50	111.57±0.32	68.64±0.58	78.46±0.44
	0.75	94.38±0.22	58.92±0.44	64.77±0.82
	1.00	64.46±0.22	46.63±0.44	53.62±0.82
T.M.	0.05	122.44±0.42	94.42±0.60	99.69±0.29
	0.10	112.46±0.47	86.58±064	78.68±076
	0.50	104.37±0.21	69.54±0.82	78.68±76
	0.75	97.83±0.14	64.77±0.64	64.99±0.84
	1.00	68.83±0.13	50.78±0.92	56.26±0.67
	Control	132.64±0.82	99.86±1.83	103.65±1.28

increase with lowering of the concentration of the dipel and it depended on the concentration ($P<0.01$)(Table – 2).

4.1A.IIb. Effect of dipel on biomass acquisition in pupae and adults under topical method:

The pupae obtained from the untreated parents acquired 132.64 mg biomass which was considerably more than that of the pupa obtained from the parents treated by topical method with any concentration of the dipel ($P<0.01$). In response to topical treatment of adults with different concentrations of dipel, the weight of the pupa varied from 68.83 to 122.44 mg and it was detected to differ with the concentrations of the dipel ($P<0.01$). In this respect, data revealed that the acquisition of the biomass in pupa declined with increasing concentrations of dipel (Table -2; Fig.-4).

The male obtained from adults, not treated topically with dipel, was heavier (99.87 mg) than that obtained from adults treated topically with any concentration of the dipel. In response to exposure of parents to residue film of different concentrations of the dipel, the male weighed 50.78 to 94.42 mg and it appeared to decrease in biomass with increase in the concentration of dipel ($P<0.01$)(Table -2).

The female obtained from the untreated adults acquired more biomass (103.65 mg) than that obtained from adults treated topically with any concentration of the dipel ($P<.0.01$). As regards the effect of the residue film of different concentrations of the dipel, the biomass accumulated by the female

varied from 56.26 to 99.69 mg, decreasing with the increasing concentration of dipel and the analysis of variance test revealed it to be dependent on the concentration of the residue film ($P<0.01$) (Table – 2).

Any concentration of the dipel applied by leaf dip method caused more decline in biomass of pupa, male adult and female adult than the same applied by topical method. So it was observed that leaf dip method was more effective than topical method.

4.1.B. Effect of thuricide on growth:

4.1.B.a Effect of thuricide on biomass accumulation in larva under leaf dip method:

The 5th day larva, not treated earlier, obtained more biomass (4.28 mg) than that obtained in response to treatments earlier with any concentration of thuricide ($P<0.05$). On this day the biomass of the treated larva vareid from 1.84 to 3.92 mg among different concentrations of the thuricide tending to decline with rise in the concentration of the thuricide. Different concentrations caused considerable reduction in the larval biomass from 1.84 to 3.92 mg significantly ($P<0.05$) (Table – 3 ; Fig. -2).

On the 10th day, the control larva acquired more biomass (22.68 mg) than that acquired by the larva obtained from earlier treated stock with any of the employed concentration of the thuricide ($P<0.05$). On this day in response to earlier treatment with the thuricide, the larval biomass varied from 7.26 to 16.78

mg among different concentrations decreasing with increase in the concentration and it differed with strength of the thuricide significantly ($P<0.05$) (Table – 3).

On the 15th day the larva not treated earlier at adult stage, had more weight (99.90 mg) than that of the larva obtained from earlier treated stock with all of the employed concentrations of the thuricide ($P<0.01$). Under this method of treatment the larval biomass varied from 24.58 to 88.46 mg among different concentrations of the thuricide and it was reduced with the increasing strength of the thuricide. The analysis of variance showed that it was affected by the strength of the thuricide significantly ($P<0.01$) (Table -3).

4.1.B.b. Effect of thuricide on biomass accumulation in larva under topical method:

The biomass accumulation in larva on 5th or 10th or 15th day, in case of its adults, not treated earlier with the residue film of the thuricide was considerably more than that in it whose adults were treated with the residue film of any concentration of the thuricide ($P<0.01$). On the 5th day, the weight of the larva, in response to adults treatment with the residue film of different concentrations of the thuricide, varied from 2.00 to 3.86 mg and tended to decrease with the increasing strengths of this bacterial preparation. As per analysis of variance, the biomass of larva differed from concentration to concentration with great significance ($P<0.01$)(Fig. -2).

In response to adults treatment with residue films of different concentrations of the thuricide, the larval weight on the 10th day varied from 9.89

to 18.67 mg and it differed with the strength of the residue film ($P<0.05$), declining with the increasing strength of the thuricide. On the 10th day, the larva of control experiment had significantly more biomass (22.68 mg) (Table -3).

Like wise on the 15th day also, the biomass of the larva of the control experiment was much heavier (99.90 mg) than that of the adults treated with the residue film of any concentration of the thuricide ($P<0.01$). On this day, in response to adults treatment with residue films of the different concentrations of the thuricide, the biomass of larva ranged from 28.68 to 90.43 mg and it differed with the concentration of the residue film significantly ($P<0.01$).

4.II.B. Effect of thuricide on biomass acquisition in pupae and adults:

4.II.B.a. Effect of thuricide on biomass acquisition in pupae and adults under leaf dip method:

The adults not treated with thuricide, produced significantly heavier pupa (132.64 mg) than those ingesting any concentration of the thuricide ($P<0.01$). In response to earlier treatment with different concentrations of thuricide the pupal biomass varied from 66.44 to 130.47 mg, decreasing with the increasing concentration and it differed significantly with the concentration of bacterial preparation ($P<0.01$)(Table – 4).

The male adult of the untreated parents, possessed more biomass (99.86 mg) than that of the parents treated earlier with any concentration of

Table - 3

Effect of different concentrations of "Thuricide" under different modes of treatment on biomass accumulation in larva of *D. obliqua*.

(Values are mean ± S.E.)

Mode of treatment	Concentration (%)	Larval biomass (mg) ± S.E. on		
		5th day	10th day	15th day
L.D.M.	0.05	3. 92±0.20	16.78±0.25	88.46±0.63
	0.10	3.18±0.14	13.94±0.43	74.64±0.56
	0.50	2.88±0.15	12.86±0.18	56.46±0.36
	0.75	2.22±0.16	10.61±0.10	30.34±0.24
	1.00	1.84±0.16	7.26±0.44	24.58±0.28
T.M.	0.05	3.86±0.26	18.67±0.14	90.43±0.36
	0.10	3.86±0.13	16.62±0.26	76.38±0.16
	0.50	2.94±0.14	13.24±0.16	57.34±0.24
	0.75	2.24±0.16	12.62±0.18	34.24±0.44
	1.00	2.00 ±0.10	9.89±0.16	28.68±0.45
	Control	4.28±0.16	22.68±0,52	99.90±0.62

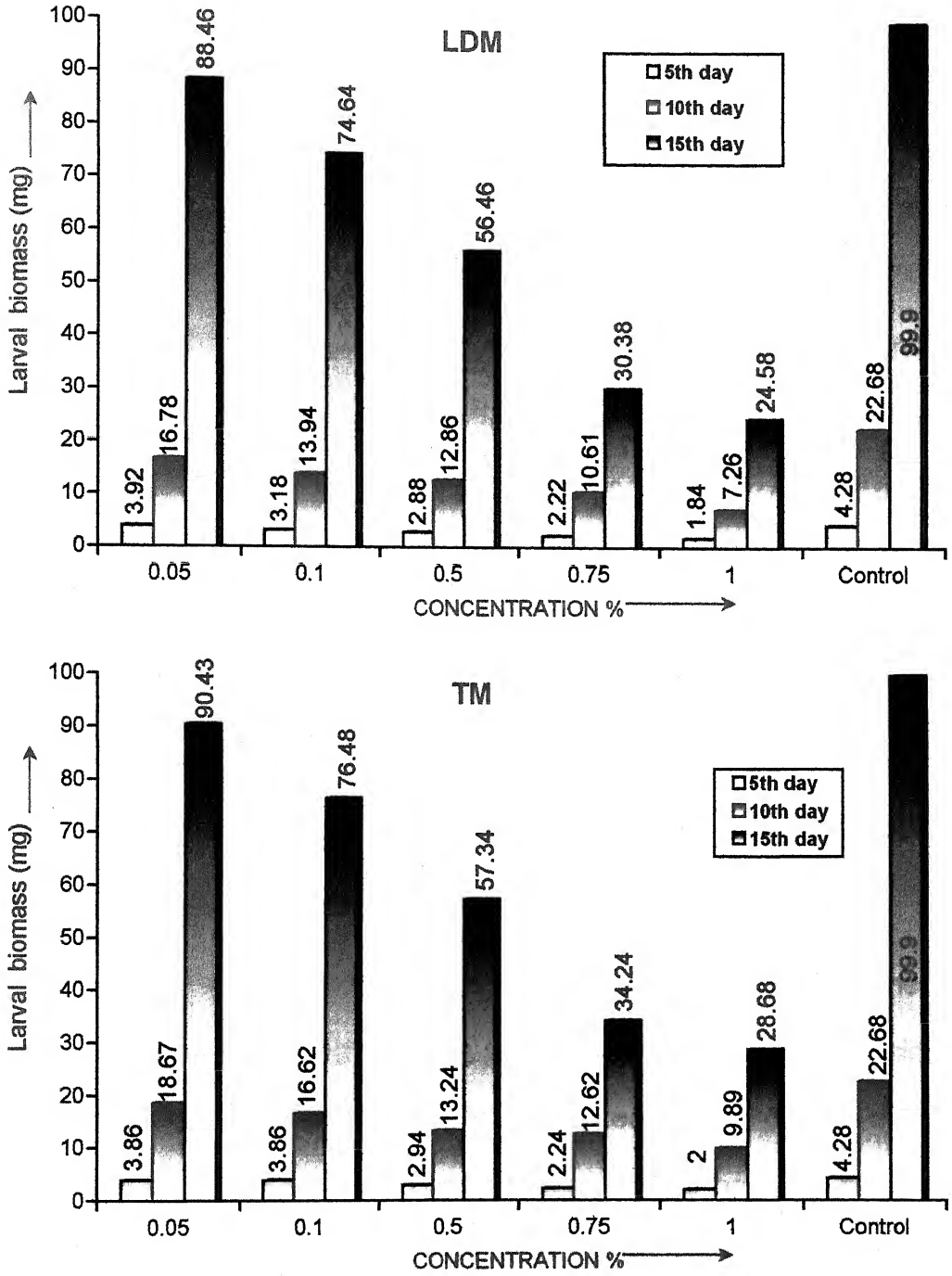


Figure 2. Effect of different concentrations of "Thuricide" under different modes of treatment on biomass accumulation in larva of *D. obliqua* on 5th, 10th and 15th day.

thuricide (P<0.01). In response to earlier treatment with different concentrations of this biological preparation, the adult male's weight varied from 49.32 to 92.43 mg, reducing with the increasing concentration and according to analysis of variance it differed from concentration to concentration significantly (P<0.01).

The female adult whose parents were not treated with the thuricide, possessed more biomass (103.65 mg) as compared to those treated earlier with any concentration of this biological preparation (P<0.01). In response to its treatment by leaf dip method with different concentrations of the thuricide, the adult female's biomass, decreasing with the increasing concentration, varied from 58.25 to 98.52 mg and it was found to depend on the concentration of the thuricide significantly (P<0.05) (Table – 4 ; Fig. 4).

4.IIB.b. Effect of thuricide on biomass acquisition in pupae and adults under topical method:

Parents treated topically with any concentration of the thuricide, produced considerably lighter pupa than those not treated with residue film of any concentration of the thuricide (P<0.01). In response to parents treatment with the residue films of different concentrations of this bacterial preparation, the biomass of the pupa varied from 69.72 to 132.48 mg, decreasing progressively with the increasing concentration and the Anova test showed that it depended on the concentration of the residue film of this bacterial preparation significantly (P<0.01) (Table – 4).

Table - 4

Effect of "Thuricide" at different concentrations under different modes of treatment on biomass accumulation by pupa and adults of *D. obliqua*.
(Values are mean ± S.E.)

Mode of treatment	Concentration (%)	Weight (mg) ± S.E. of		
		Pupa	Male	Female
L.D.M.	0.05	130.47±0.33	92.43±0.44	98.52±0.53
	0.10	124.62±0.44	79.48±0.75	86.54±0.42
	0.50	114.62±0.32	69.36±0.56	74.48±0.42
	0.75	96.34±0.24	60.42±0.66	64.62±0.84
	1.00	66.44±0.23	49.32±0.45	58.25±0.83
T.M.	0.05	132.48±0.82	95.66±0.76	100.88±0.35
	0.10	128.56±0.36	88.52±0.62	89.56±0.64
	0.50	116.47±0.92	78.65±0.54	76.68±0.62
	0.75	99.78±0.85	64.58±0.56	66.92±0.85
	1.00	69.72±0.63	52.48±0.42	60.48±0.55
	Control	132.64±0.82	99.86±1.89	103.65±1.28

The male adult of the untreated parents acquired considerably more biomass (99.86 mg) as compared that of parents treated with any concentration of the thuricide under topical method ($P<0.01$). As regards the influence of the parents treated by topical method with different concentrations of the thuricide on the acquisition of biomass in male adult, its biomass varied from 52.48 to 95.66 mg, showing a tendency towards decrease with the increasing concentration and it was detected to differ from concentration to concentration significantly ($P<0.01$) (Table – 4 ; Fig. - 4).

The parents treated with the residue film of any concentration of the thuricide produced considerably lighter female than the untreated parents (103.65 mg). In response to the parents treatment with residue films of different strengths of the thuricide (0.05% to 1.0%), the biomass of female adult varies from 60.48 to 100.88 mg, tending to decrease with the increase in the concentration and as per statistical analysis, biomass differed from concentration to concentration significantly ($P<0.01$) (Table – 4).

4.1C. Effect of bactospeine on growth:

4.1C.a. Effect of bactospeine on biomass accumulation in larva under leaf dip method:

The larva of the adults treated earlier with different concentrations of the bactospeine had lesser biomass on fifth day as compared that of the untreated adults (4.28 mg). The larval biomass on fifth day, in response to the

treatment with different effective concentrations (0.05 to 1.0%) of this bacterial preparation, varied from 2.56 to 4.15 mg, decreasing with the increasing concentration of the biopesticide ($P<0.01$) (Table – 5).

On the 10th day, the larva of adults treated orally with any concentration of the bactospeine was considerably lighter than that of the untreated parents ($P<0.01$). In response to earlier treatment with different concentrations of this bacterial preparation, the larval biomass on this day, showing a tendency towards decrease with the advancing concentration, varied from 8.74 to 21.68 mg and it depended on the concentration of the bactospeine significantly ($P<0.01$) (Fig. -3).

Like the 10th day, on the 15th day also, each concentration of the bactospeine under leaf dip mehtod reduced the larval biomass as compared the non treatment situation ($P<0.01$). As regards the effect of the different concentrations of this bacterial preparation, the larval biomass on this day, tending to decrease with the advancing concentration, varied from 24.28 to 88.48 mg and, as per analysis of variance, it differed with the concentration of the bactospeine significantly ($P<0.01$) (Table – 5).

4.1C.b. Effect of bactospeine on biomass accumulation in larva under topical method:

On the fifth day, the larva of the parents treated topically with any concentration of the bactospeine was considerably lighter than that of the untreated parents ($P<0.01$). In response to parents treatment with the residue

Table - 5

Effect of different concentrations of "Bactospeine" under different modes of treatment on biomass accumulation in larva of *D. obliqua*.

(Values are mean ± S.E.)

Mode of treatment	Concentration (%)	Larval biomass (mg) ± S.E. on		
		5th day	10th day	15th day
L.D.M.	0.05	4.15±0.16	21.68±0.25	88.48±0.45
	0.10	3.93±0.24	16.47±0.31	74.44±0.48
	0.50	2.96±0.18	14.18±0.18	54.47±0.48
	0.75	2.68±0.22	11.14±0.22	36.47±0.17
	1.00	2.06±0.24	8.74±0.17	24.28±0.46
T.M.	0.05	3.94±0.38	22.24±0.16	89.47±0.28
	0.10	3.84±0.14	16.47±0.24	76.46±0.18
	0.50	3.87±0.17	14.46±0.11	48.44±0.26
	0.75	2.29±0.14	12.84±0.11	35.38±0.19
	1.00	2.18±0.27	9.09±0.10	25.26±0.25
	Control	4.28±0.16	22.68±0.52	99.90±0.62

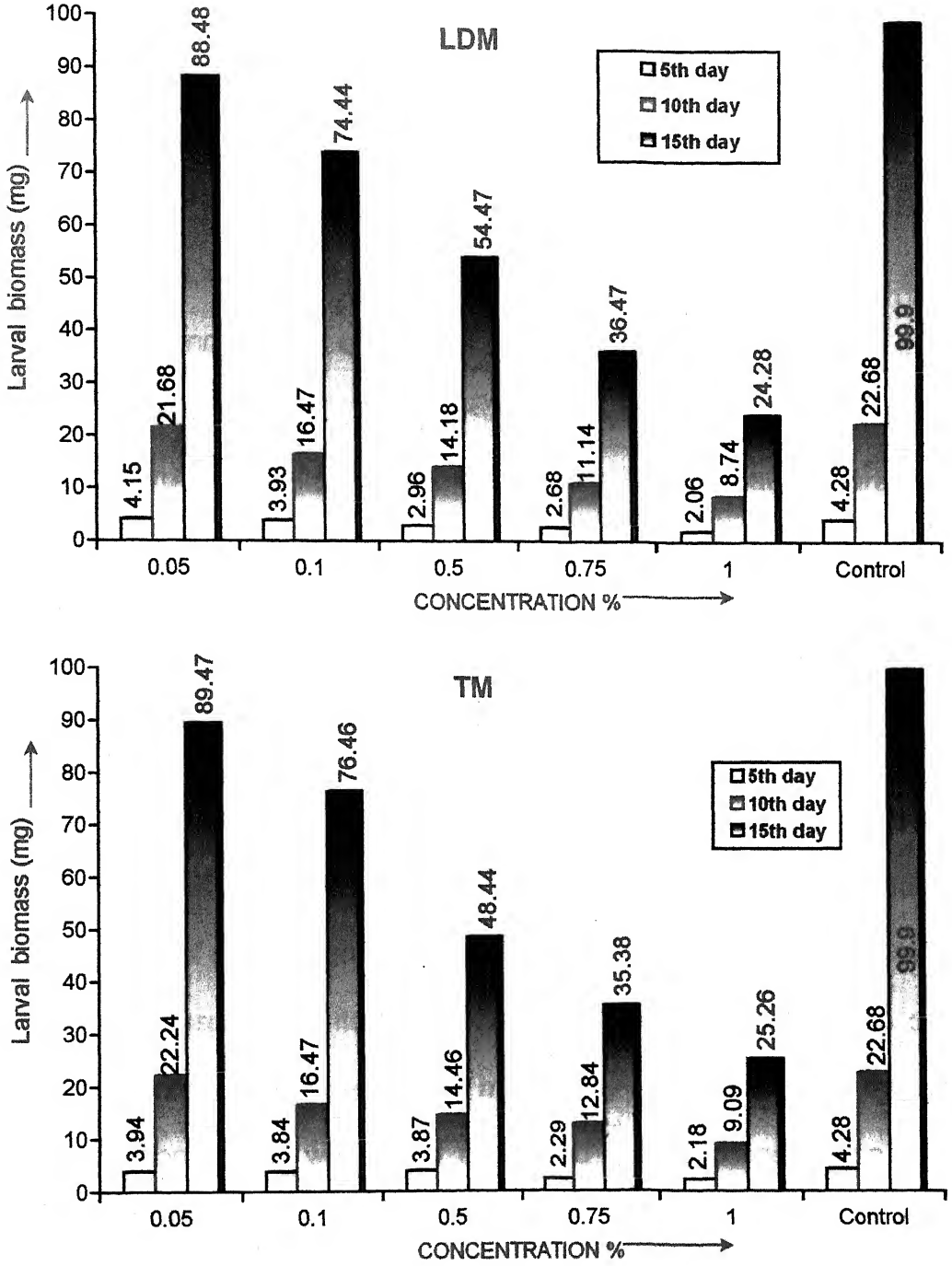


Figure 3. Effect of different concentrations of "Bactospeine" under different modes of treatment on biomass accumulation in larva of *D. obliqua* on 5th, 10th and 15th day.

films of different concentrations of bactospeine the larval biomass on this day, decreasing with the increasing concentration, varied from 2.18 to 3.94 mg, significantly ($P<0.01$) (Table – 5 ; Fig. - 3).

On the tenth day, the larva of the parents treated with the residue film of any concentration of the bactospeine accumulated less mass than that of the untreated parents (22.68 mg, $P<0.01$). The treatment of the parents with the residue films of different concentrations of this bacterial preparation caused variation in the larval biomass (9.09 to 22.24 mg) among different concentrations with a tendency towards decrease with the advancing concentration and as per analysis of variance, it differed from concentration to concentration significantly ($P<0.01$) (Table – 5).

On the 15^{th} day also, the larva of the untreated adults had more biomass (99.90 mg) than that of the adults treated with the residue film of bactospeine of any concentration ($P<0.01$). The larva acquired 25.26 to 89.47 mg biomass in response to its parents treatment with the residue films of different concentrations of bactospeine with a tendency of reduction with the increasing concentration and, it was found to depend on the concentration significantly (Anova, $P<0.01$) (Table – 5 ; Fig. 3).

4.1C.II. Effect of bactospeine on acquisition of biomass in pupae and adults:

4.1C.IIa. Effect of bactospeine on acquisition of biomass in pupae and adults under leaf dip method:

The pupa of the larvae treated orally with any concentration of the bactospeine was lighter (132.64 mg) than of the untreated adults ($P<0.01$). In response to its treatment by leaf dip method with different strengths of bactospeine, the pupa acquired 68.24 to 132.58 mg biomass, decreasing with the increasing concentration, differed from strength to strength of this bacterial preparation significantly ($P<0.01$) (Table – 6).

The male adult of the untreated parents acquired considerably more biomass (99.86 mg) as compared to that of the larvae treated with any concentration of the bactospeine by leaf dip mehtod ($P<0.01$). In response to treatment with different concentrations under leaf dip method, the biomass of male adult varied from 59.28 to 94.59 mg, showing a tendency towards decrease with the increasing concentration and it was detected to differ from concentration to concentration significantly ($P<0.01$) (Table – 6).

The female adult of the untreated parents had more biomass (103.65 mg) than that of the larvae treated orally with any strength of this bactospeine ($P<0.01$). Varying from 62.46 to 100.63 mg among different concentrations of this bacterial preparation and tending to decrease with the

advancing concentration, the female adults biomass depended on the strength of the bactospeine under leaf dip method (Anova, $P<0.01$).

4.1.C.IIb. Effect of bactospeine on acquisition of biomass in pupae and adults under topical method:

Parents treated topically with any concentration of the bactospeine, produced considerably lighter pupa than those not treated with residue film of any concentration of this bacterial preparation ($P<0.01$). In response to parents treatment with the residue films of different concentrations of bactospeine, the biomass of the pupa varied from 70.45 to 134.68 mg, decreasing progressively with the increasing concentration and the Anova test showed that it depended on the concentration of the residue film of this bacterial preparation ($P<0.01$).

The male adult whose parents were not treated topically with bactospeine, acquired considerably more biomass (99.86 mg) than that whose parents were treated with the residue film of any concentration of bactospeine ($P<0.01$). As regards the effect of parents treatment with the residue films of different strengths of the bactospeine on the acquisition of biomass in male adult, the weight of the male adult varied from 62.47 to 99.72 mg, showing a tendency towards decrease with the increase in the concentration of the residue film and as per statistical analysis, it depended on the concentration of the residue film of the bactospeine ($P<0.01$) (Table – 6 ; Fig. - 4).

Like the male adult, the female adult of the untreated parents also acquired more biomass than that of those treated with the residue film of any

Table - 6

Effect of "Bactospeine" at different concentrations under different modes of treatment on biomass accumulation by pupa and adults of *D. obliqua*.
(Values are mean ± S.E.)

Mode of treatment	Concentration (%)	Weight (mg) ± S.E. of		
		Pupa	Male	Female
L.D.M.	0.05	132.58±0.48	94.59±0.25	100.63±0.42
	0.10	125.64±0.44	80.56±0.05	86.14±.0.44
	0.50	115.72±0.45	70.47±0.62	76.78±0.72
	0.75	98.08±0.84	62.47±0.58	65.76±0.63
	1.00	68.24±0.85	59.28±0.48	62.46±1.65
T.M.	0.05	134.68±0.82	99.72±0.85	100.82±0.46
	0.10	128.47±0.62	88.36±0.63	90.58±0.46
	0.50	118.33±0.62	77.68±0.62	78.68±0.74
	0.75	100.47±0.44	64.41±0.38	67.38±0.63
	1.00	70.45±0.34	62.47±0.16	68.42±0.42
	Control	132.64±0.82	99.86±1.89	103.65±1.28

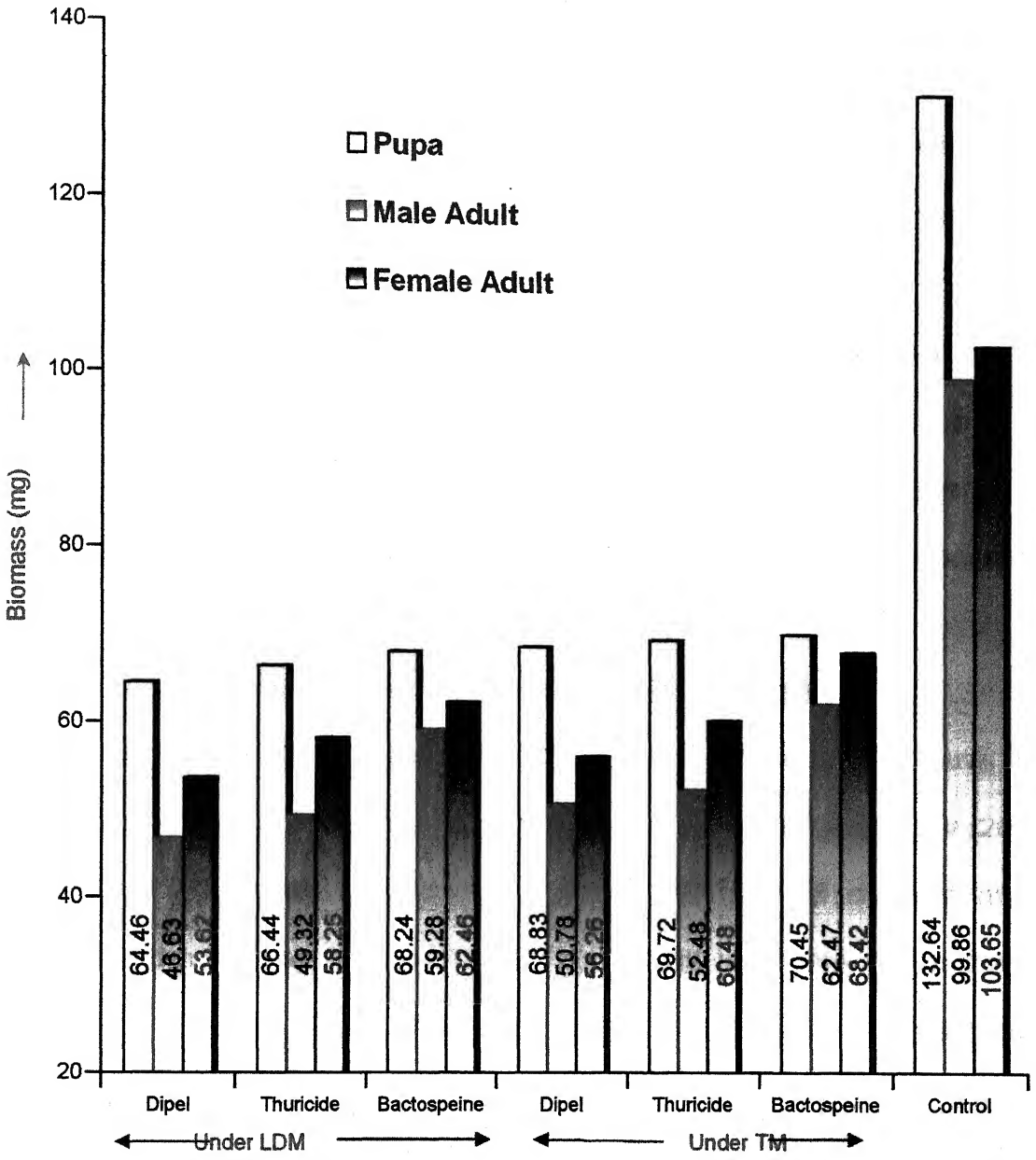


Figure 4. Effect of different biological preparations (one per cent) under LDM and TM on biomass accumulation by pupa, male and female adults of *D. obliqua.*

concentration of the bactospeine (P<0.01). Further, the weight of the adult female varied form 68.42 to 100.82 mg in response to treatment with residue films of different concentrations of this bacterial preparation and it differed with the strength of residue film (P<0.01). The acquisition of the biomass was inversely proportional to the strength of the bactospeine (Table – 6 ; Fig. -4).

As regards the influence of the bacterial preparation on the biomass accumulation in *D. obliqua* larva, the related results have shown that every bacterial preparation (dipel, thuricide, bactospeine) under this investigation has potential to reduce the growth in this insect even at a very low concentration (0.05). Krieg (1961), Heimpel (1967).Sundrababu and Subramaniam (1973). Dobrivojevic & Injac (1975), Moawad *et. al.* (1985), Svestka, (1975), Srivastava and Nayak (1978) Srivastava and Ramakrsihnan (1980), Srivastava (1984) Chaturvedi (2003) , Naglaa *et al.* (2004), Bakr (2004), Kumar & Gujar (2005), Li *et. al.* (2005) have also observed similar influence of different microbial insecticides in other insects. Further, our results have also shown that in *D. obliqua*, the effect of the different concentrations of a microbial insecticide on accumulation of the biomass in the larva, which may not be graded in early larval life, becomes quite distinct in the late larva; the biomass reducing potential of dipel, thuricide and bactospeine increases with the increase in its concentration.

Further, in respect of the influence of microbial insecticides on the biomass accumulation in *D. obliqua* under both modes of application reduce the larval biomass almost identically from early to late larval life at different

concentrations. The microbial insecticides applied by the leaf dip method reduced the biomass of larva more than when it is administered by topical method. The corresponding concentrations are equally effective in reducing the larval biomass under topical method as compared the application of the same as the leaf dip method.

However, different concentrations of dipel, thuricide, and bactospeine proved their toxic effect in declining the biomass of the larvae when they administered by leaf dip method or topically, but with the leaf dip method, dipel becomes most effective than applied by topical method to the adult. The fact that all microbial insecticides used in this investigation are equally effective in reducing the accumulation of biomass in the larva on 5^{th}, 10^{th} and 15^{th} day.

Since adequate growth is an attribute of proper nutritional metabolism, it may be presumed that the above mentioned microbial insecticides interfere the aspect of physiology and morphology in *D. obliqua* hence they reduce the accumulation of the biomass in larvae of this moth. Herbert and Harper (1985) has reported that the apholate accepts amino acids as ligands, binding to NH_2 site and consequently, inhibiting formation of the peptide linkage, it reduces the synthesis of some proteins in *Diaphania nilidalis* which owing to the same, exhibits poor growth. In this moth also, the used microbial insecticides may hinder the protein synthesis causing consequent reduction in the larval biomass.

The weight loss in larva on 5th, 10th and 15th day was recorded at each tested level. It was observed, that there was a regular increase in weight loss from the lowest concentration (0.05%) to the highest (1.0%). Loss of weight of larvae might be due to the irritating action of the microbial insecticide, resulting in water loss from the body. Many workers reported greater amount of weight loss in larvae due to treatment with different microbial insecticides in different insects.

In context of the efficiency of the microbial insecticides in reducing the accumulation of the biomass in larvae, as per result of this investigation, considering concentrations from 0.05% to 1.0%, the three microbial insecticides screened under this investigation may be arranged as dipel, thuricide and bactospeine.

Like the accumulation of the biomass in the larva, the acquisition of biomass in pupa and adults is also reduced by every microbial insecticide under leaf dip method and topical method. Both methods are effective in causing reduction in the biomass of pupa and adults. Administering dipel orally at larval stage causes more reduction in the biomass of pupa, male and female *D. obliqua* than treatment under topical treatment. At 1.00% concentration all three microbial preparations caused more reduction in pupa and adults under leaf dip treatment.

Further, the results in this context reveal that there is an indirect proportionality between the biomass of these stages of life cycle and concentrations of microbial preparations. As regards the pupal biomass reducing

potential of the different microbial insecticides, these microbial preparations can be arranged as dipel, thuricide, and bactospeine in descending order. Results also reveal that these microbial insecticides are identically effective in both sexes. Regarding the weight loss, these findings support, the views of Hall and Arakawa (1959), Hall et. al. (1960), Mc. Even et. al. (1960), Smirnoff (1981) Mc Leod et. al. (1982), Bosgelmen *et. al.* (1984).

Enough literatures are not available on the weight loss in insects during treatment period with the pathogens. However, literatures are available on weight loss during exposure period with different insecticides. Since the pathogen *B. thuringiensis* also causes poisonous symptoms like insecticides, it may be compared with them. Singh *et.al.*(1985) studied the weight and water loss of *Achoea janata* larvae with different dosages of insecticides. Treated larvae excreted a greater amount of the excreta and lost water faster than control test. These findings have further been substantiated by the study of Srivastava and Nayak (1978) against *C.medinalis*; Srivastava (1984) against *P. demoleus,* Sundrababu and Subramanium (1973) against larvae of *S. litura*. The findings of this investigaiton also gets support from Krieg (1961) and Heimpel (1967) who reported variation in toxicity due to different varities of *B. thuringiensis* against different insect species tested by them. Similar results were also reported by Srivastava (1984) against the larvae of *P. demoleus*. Dipel used as controlling agent against other lepidopterous insects have also been reported by Dobrivojevic and Injac (1975) and Moawad *et. al.* (1985), Chaturvedi (2003),

Bajpai (2003) Devaki and Krishnayya (2004), Hernadez *et. al.*(2005), Gopalakrishnan and Gangavisalakshy (2005), Li *et. al.*(2005) which further strengthen the findings of this investigation.

As regards larval weight of treated insects, Narayan and Jayraj (1975) observed an abatement of 46.1 per cent in weight in the case of *P. demoleus*. This is incomplete agreement with the result of present investigation. However, contrary to findings of earlier workers Fast and Regniere (1984) did not find any difference in the weight, gained or lost by the treated insects while comparing with control. In the present study, reduction in weight of *D. obliqua* larvae and adults is possibly due to the cessation of feeding at the initial stage.

As regards the effect of bacterial treatment on the larval mortality of *D. obliqua,* Govindrajan *et. al.* (1975) observed this phenomenon at the pre pupal stage of *S. litura* after the treatment with thuricide. In the present study too, author has also recorded the larval mortality at the advance stage of *D. obliqua*.

Consultation of literature on the effect of *Bt.* on pupal development reveals that the resultant pupae from the bacterial infected larvae exhibited an adverse effect on their duration, size, weight and survival. Sareen *et. al.* (1983) in *S. litura*, Fast and Dimond (1984) in C. fumiferana and Chaturvedi (2003) in *Utetheisa pulchella* reported the reduction in pupal weight after intoxication with *B. thuringiensis*. They further added that declination in weight of treated larvae was closely related with the concentration of pathogen used.

As regards the effect of bacterial treatment on pupal weight is concerned, Abdul Sattar and Watson (1982) and Luttrel *et. al.* (1982) and Bajpai (2003) observed an increase in pupal period of *H. virescens* after exposure to the bacterium. The findings of the present investigation are in full alignment with the reports of earlier workers.

4.2. EFFECT OF BACTERIAL PREPARATIONS ON POST – EMBRYONIC DEVELOPMENT:

4.2.A. Effect of dipel on post embryonic development:

4.2.A.a. Effect of dipel on post embryonic development under leaf dip method:

The larva of the untreated adults acquired 89.43 per cent survival, whereas that of the larvae treated orally with any concentration acquired survival in the range between 22.14 and 65.35 per cent ($P<0.05$). In response to treatment by leaf dip method with different concentrations of the bacterial preparation (dipel) results showed that the larval survival, varying from 22.14 to 64.36 per cent and decreasing with the advancing concentration, depended on the concentration of the dipel ($P<0.05$). Further, the larva of the untreated adults grew faster than that of the adults treated with any concentration of the dipel ($P<0.01$) (Table – 7).

As regards influence of different concentrations of dipel administered by leaf dip method, the duration of larval stage, varying from 22.65

Table - 7

Effect of "Dipel"on post embryonic development in *D. obliqua* at different concentrations under different modes of treatment.

(Values are mean ± S.E.)

Mode of treatment	Concentration (%)	Pupation (%)	Larval period (days)	Emergence (%)	Pupal period (days)
L.D.M.	0.05	65.35	22.65±0.68	70.62	14.52±0.56
	0.10	57.38	24.85±0.26	60.54	16.76±0.75
	0.50	44.44	28.47±0.25	40.34	20.64±0.31
	0.75	35.26	34.24±0.26	35.27	24.44±0.56
	1.00	22.14	36.42±0.44	22.28	29.18±0.91
T.M.	0.05	70.65	24.42±0.54	70.74	16.17±0.88
	0.10	58.37	25.42±0.84	60.83	17.72±0.94
	0.50	45.26	26.72±0.72	41.37	20.84±0.44
	0.75	36.68	30.57±0.77	38.33	24.52±0.14
	1.00	25.42	40.43±0.67	25.74	30.41±0.48
	Control	89.43	16.35±0.42	100.00	12.76±032

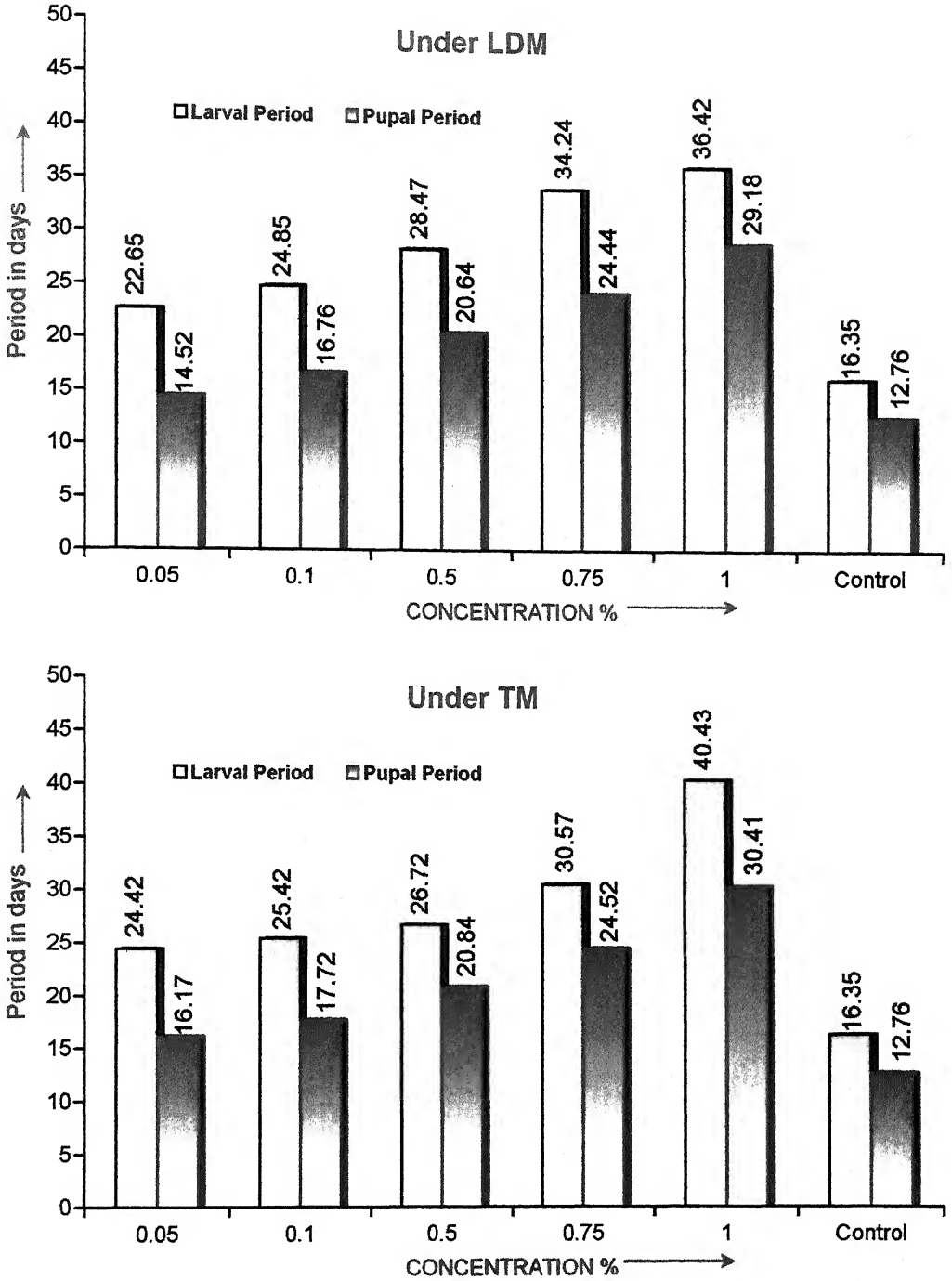
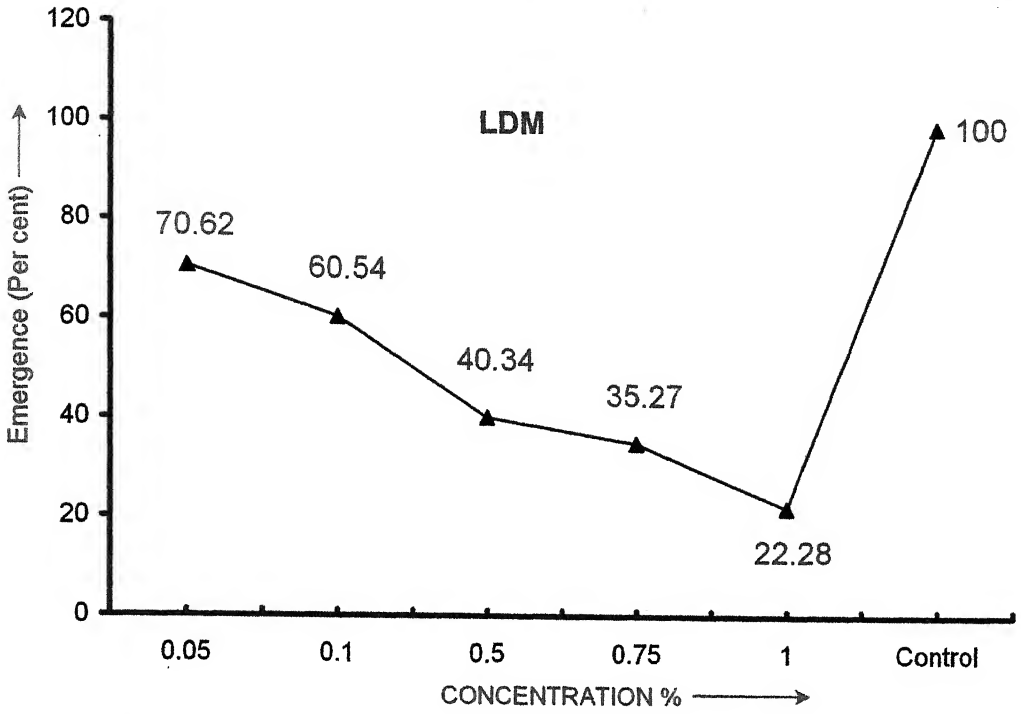


Figure 5. Effect of different concentrations of "Dipel" on larval and pupal period of *D. obliqua* under LDM and TM of treatment.



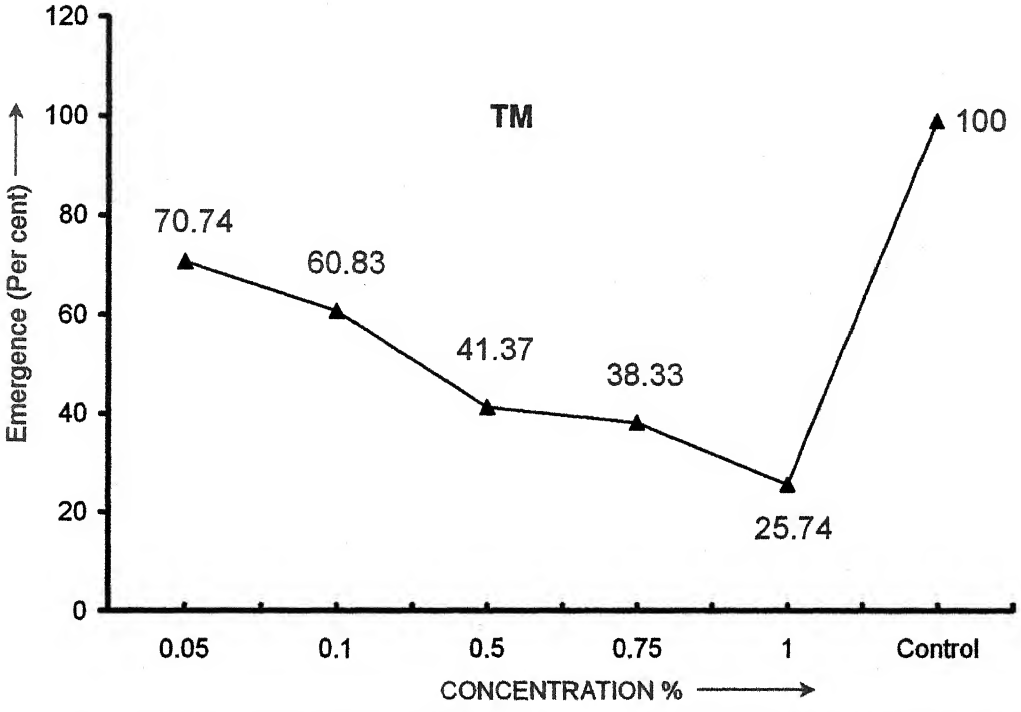


Figure 6. Effect of "Dipel" on emergence in *D. obliqua* at different concentrations under LDM and TM modes of treatment.

to 36.42 days and increasing with the increasing concentration of dipel, was detected to depend on the concentration ($P<0.05$). Further, the pupa of the untreated stock had hundred per cent emergence which was much curtailed in case of the pupa of the treated stock with any concentration of this bacterial preparation. In response to treatment by leaf dip method with different concentrations of the dipel, the percentage of the emergence, varying from 22.28 to 70.62 per cent and decreasing with the increase in the concentration, was detected to differ with the concentration ($P<0.05$) (Table – 7 ; Fig. - 6).

There was no significant difference in the pupal period between the non-treatment condition and the treatment situation at 0.05 per cent ($P>0.05$) but above this strength, the pupal period was prolonged considerably by any of the remaining concentrations ($P<0.05$). In response to treatment by leaf dip method with different concentrations of dipel from 0.05 to 1.0 per cent, the pupal period, varying from 14.52 to 29.18 days and increasing with the increasing in the concentration, depended significantly on the strength of the dipel ($P<0.05$).

Further, the results pertaining to the net mortality revealed that in response to leaf dip method of treatment with different concentrations of the dipel, as per chi-square test, it varies from 52 to 96 per cent and increasing with the rise in the strength of the dipel , differed significantly with the concentration ($P<0.05$) (Table – 8 ; Fig. - 7).

The leaf dip treatment with any concentration of the dipel curtailed the longevity of both male and female adults significantly as compared to non

Table -8

Net mortality in *D. obliqua* caused by "Dipel" at different concentrations under different modes of treatment.

(Values are mean ± S.E.)

Mode of treatment	Concentration applied %	No. of larvae reared	No. of larvae died	No. of Pupae died	Total death	%net mortality
L.D.M.	0.05	60	24	12	36	52
	0.10	60	32	16	48	76
	0.50	60	35	18	53	86
	0.75	60	38	15	53	86
	1.00	60	46	12	58	96.
T.M.	0.05	60	22	16	38	56
	0.10	60	27	22	49	78
	0.50	60	35	15	50	80
	0.75	60	38	15	53	86
	1.00	60	40	14	54	88
	Control	60	10	NIL	10	—

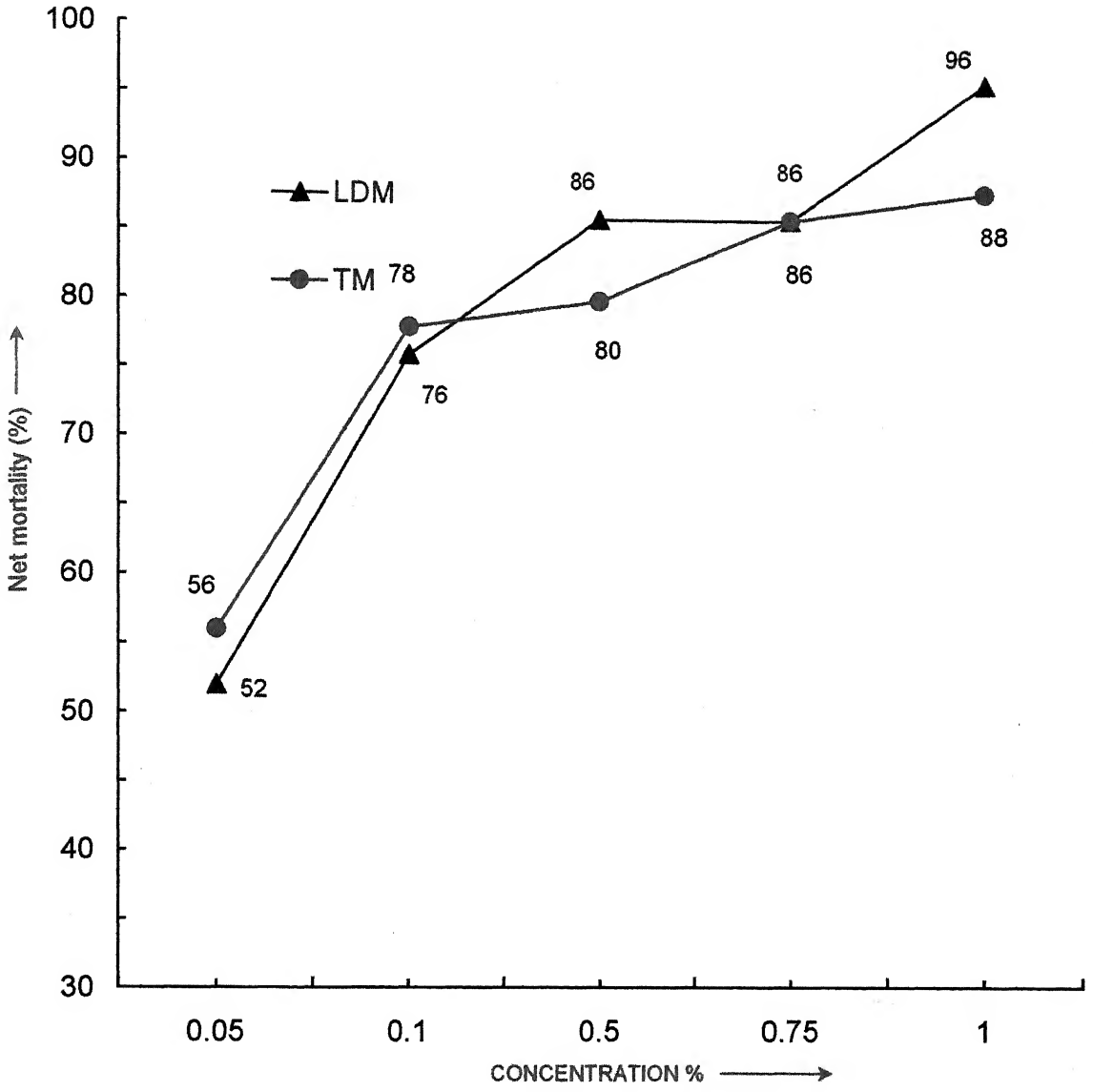


Figure 7. Net mortality in *D. obliqua* caused by "Dipel" at different concentrations under LDM and TM modes of treatment.

treatment at early stage (P<0.05). The female lived longer than the male in response to treatment with any concentration of the dipel. The life span in both sexes, decreasing with the increasing concentration, differed with the concentration of the dipel (P<.0.05) (Table -9).

4.2.A.b. Effect of dipel on post embryonic development under topical method:

The treatment of the parents with residue film of any concentration of dipel induced significantly less larval survival as compared the untreated parents (P<0.05). As per chi-square test the larval survival varying from 25.42 to 70.65 per cent among the different concentrations of the microbial preparation and decreasing with the increasing concentration depended on the concentration (P<0.05). Further, the parents residue film treatment with any concentration of the dipel prolonged the larval stage as compared the duration of the larva of the untreated parents (P<0.05). In response to parents treatment with dipel, the larval period, varying from 24.42 to 40.43 days among different concentrations and appearing to be directly proportional to the concentration, was affected significantly (P<0.05) (Table – 7).

The treatment of the parent adult with residue film of any strength of the dipel reduced emergence and prolonged the pupal period, in general, as compared the untreated conditon (P<0.05). Among residue films of different concentrations of this microbial preparation, the emregecne, varying from 26.74 to 72.75 per cent and appearing indirectly proportional to the concentration was

Table - 9

Effect of "Dipel" on longevity in male and female *D. obliqua* at different modes of treatment.

(Values are mean ± S.E.)

Mode of treatment	Concentration (%)	Longevity (In days)	
		Male	Female
L.D.M.	0.05	7.26±0.34	8.35±0.25
	0.10	7.68±0.26	7.75±0.26
	0.50	6.02±0.48	6.93±0.44
	0.75	5.06±0.78	5.18±0.56
	1.00	3.14±0.12	3.16±0.02
T.M.	0.05	7.80±0.44	9.76±0.32
	0.10	7.06±0.22	8.82±0.26
	0.50	7.36±0.34	7.87±0.16
	0.75	6.54±0.56	6.86±0.46
	1.00	3.45±0.14	3.55±0.42
	Control	11.35±0.22	13.76±0.24

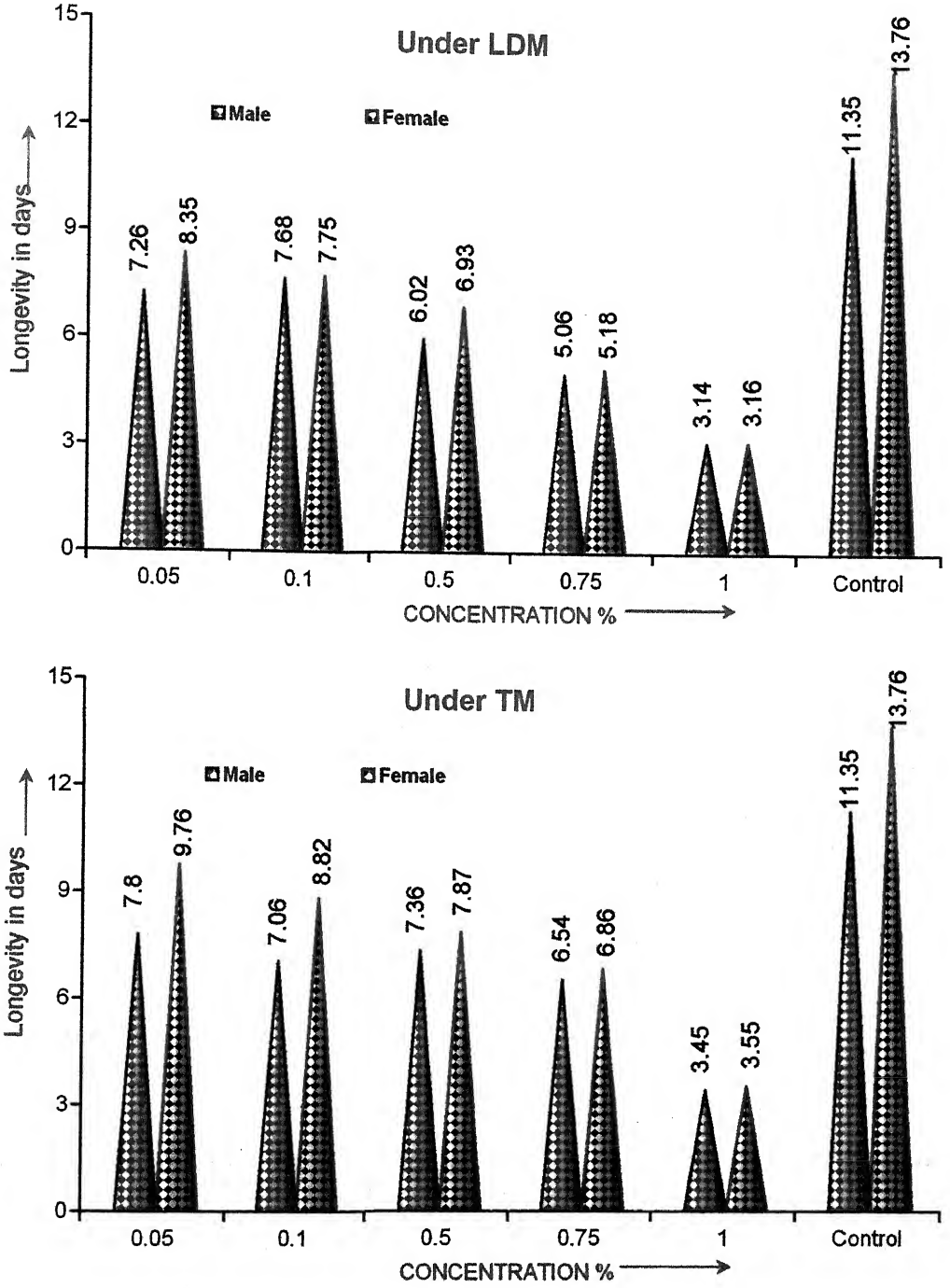


Figure 8. Effect of "Dipel" on longevity in male and female *D. obliqua* at LDM and TM modes of treatment.

detected statistically to differ with concentrations ($P<0.05$). Like wise the pupal period which varied from 16.17 to 30.14 days among residue film concentrations from 0.05 to 1.0 per cent and tended to be directly proportional to the strength of the residue film, was found to be effective differently by the residue films of different concentrations ($P<0.05$) (Table – 7).

The net mortality, varying from 56 to 88 per cent and showing direct proportionality to the concentration, differed significantly with the residue films of different concentrations of the dipel ($P<0.05$) (Table -8 Fig. -7).

The treatment of parent adults with residue film of any concentration of the dipel reduced the longevity in adults of both sexes as compared the untreated parent adults ($P<0.05$).The life span of progeny adult in either sex, varying from 3.45 to 7.80 days in male and from 3.55 to 9.76 days in female in response to residue films of different concentrations and tending to be indirectly proportional to the residue film concentration, differed significantly with the strength of the residue film of the dipel ($P<0.05$) (Table – 9 ; Fig. – 8).

4.2.B. Effect of Thuricide on post embryonic development:

4.2. B.a. Effect of thuricide on post-embryonic development under leaf dip method:

Any concentration of the thuricide applied to larvae through food, reduced the larval survival considerably as compared the untreated adults ($P<0.05$). As regards the effect of different concentrations of this bacterial

preparation on the larval survival, the percentage of pupation, varying from 25.26 to 69.42 per cent and decreasing with the increasing concentration differed from concentration to concentration as per chi-square test (Table – 10).

Further, any concentration of this microbial preparation prolonged the larval stage as compared the untreated condition ($P<0.05$) and the larval stage, varying from 22.76 to 36.46 days in response to treatment by leaf dip method and showing direct proportionality to the concentration, differed with the concentrations of thuricide ($P<0.05$) (Fig. – 9).

Every concentration of the thuricide under leaf dip method , caused considerable reduction in emergence and marked prolongation in pupal stage ($P<0.05$). Tending to decrease with the advancing concentration, the emergence, varying from 22.12 to 71.72 per cent, differed significantly with concentrations of the thuricide ($P<0.05$) (Table – 10 ; Fig. - 10).

Like the emergence, the pupal period, varying from 14.82 to 29.76 days among different concentrations of the thuricide also differed with different concentrations but it tended to be directly proportional to the concentration of the microbial preparation.

Further, the net mortality also varying from 52 to 94 per cent among different concentrations of the thuricide, differed from concentration to concentration ($P<0.05$) and it tended to decrease with the increasing concentrations significantly (Table – 11).

Table - 10

Effect of "Thuricide" on post embryonic development in *D. obliqua* at different concentrations under different modes of treatment.
(Values are mean ± S.E.)

Mode of treatment	Concentration (%)	Pupation (%)	Larval period (days)	Emergence (%)	Pupal period (days)
L.D.M.	0.05	69.42	22.76±0.68	71.72	14.82±0.42
	0.10	58.48	26.52±0.66	65.65	15.76±0.44
	0.50	48.24	28.27±0.28	54.72	19.76±0.42
	0.75	36.37	33.32±0.37	42.65	23.86±0.56
	1.00	25.26	36.46±0.72	22.12	29.76±0.40
T.M.	0.05	69.85	19.64±0.75	72.82	14.94±0.22
	0.10	58.40	24.78±0.69	68.47	15.78±0.34
	0.50	48.32	26.85±0.42	56.32	20.87±0.46
	0.75	36.72	30.75±0.17	48.11	23.60±0.06
	1.00	27.12	36.30±0.12	29.75	28.70±0.82
	Control	89.43	16.35±0.42	100.00	12.76±0.32

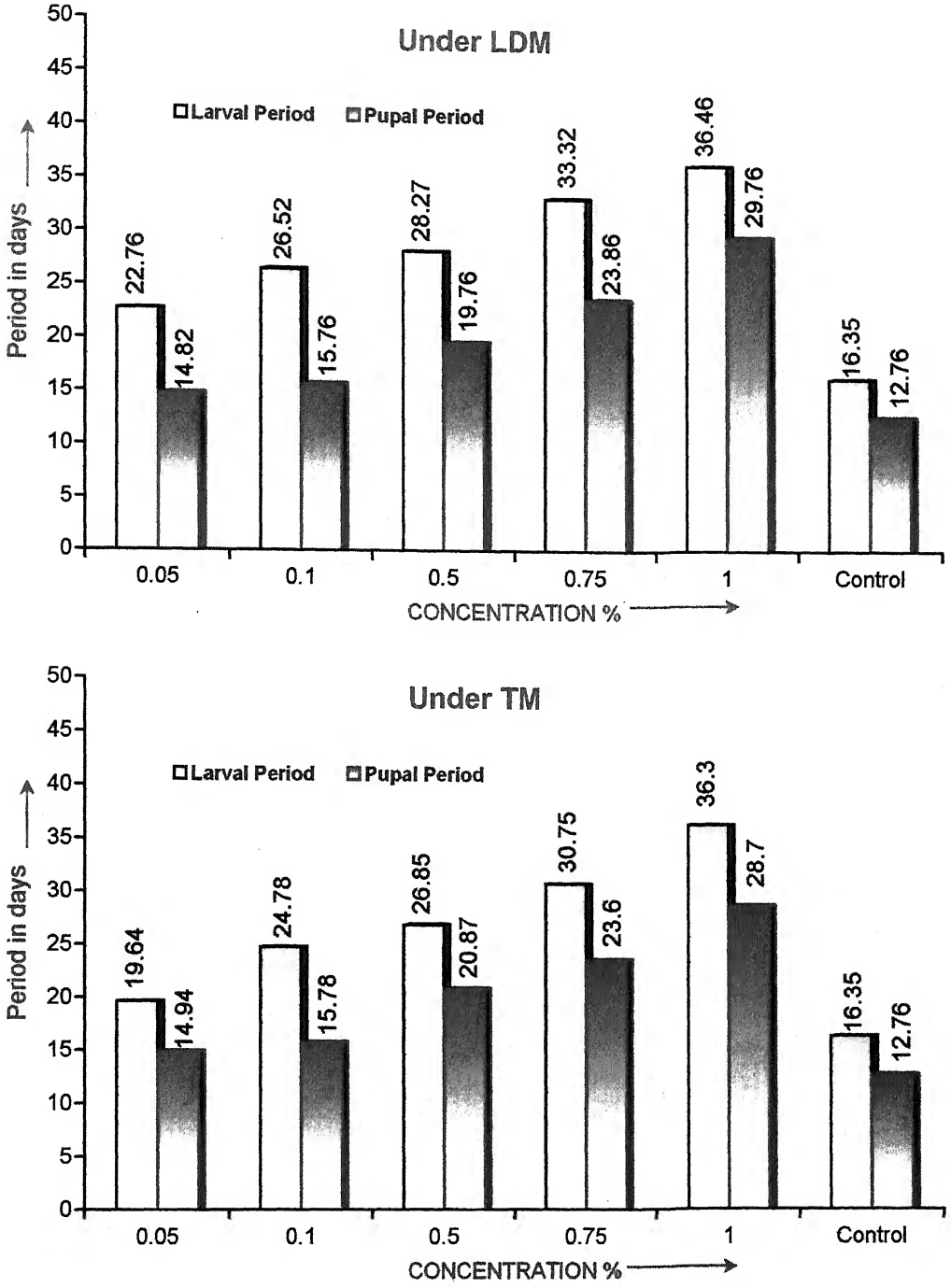
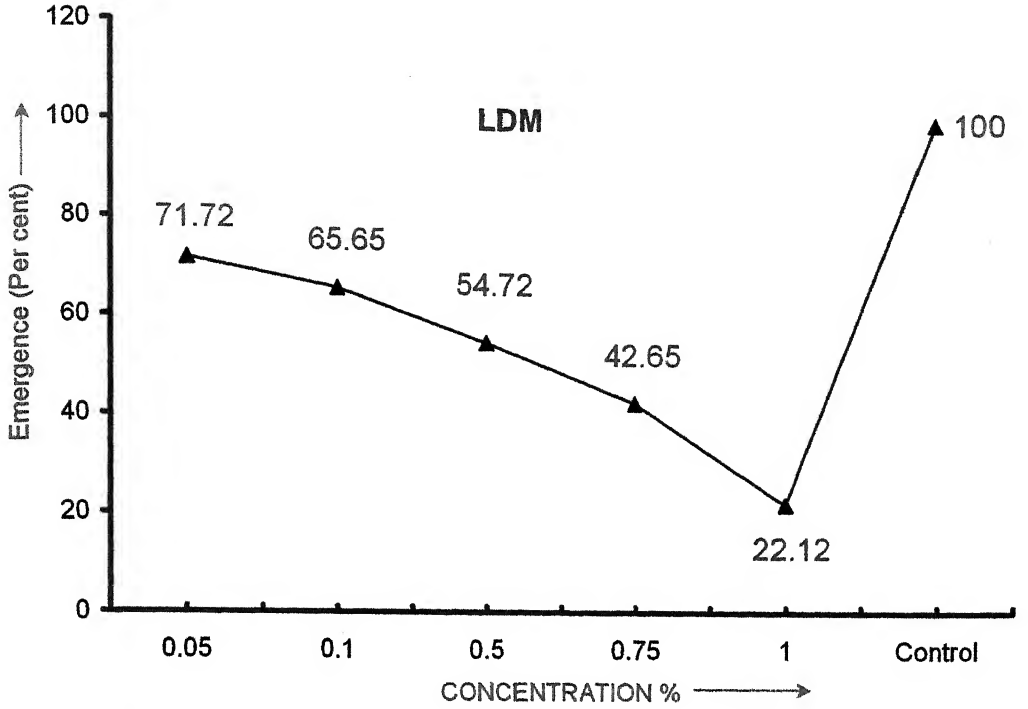


Figure 9. Effect of different concentrations of "Thuricide" on larval and pupal period of *D. obliqua* under LDM and TM of treatments.



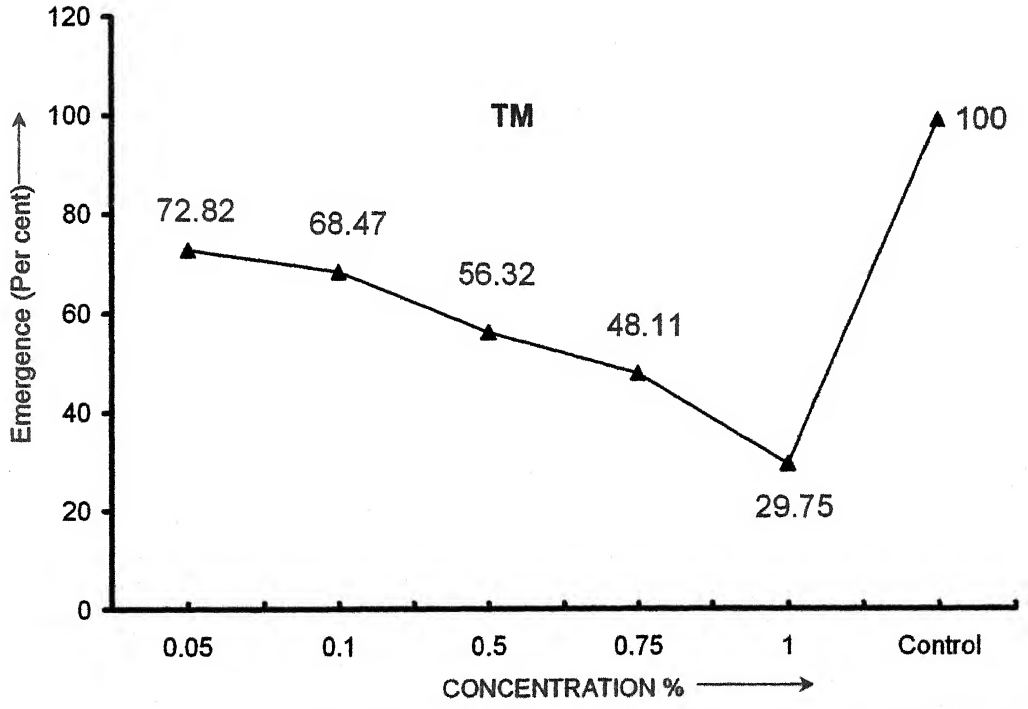


Figure 10. Effect of "Thuricide" on emergence in *D. obliqua* at different concentrations under LDM and TM modes of treatment.

The life span of progeny male and female adults of untreated parents was more as compared to that parents earlier treated by leaf dip method with any concentration of the thuricide ($P<0.05$). The longevity of adults, varying from 3.76 to 7.92 days in male and from 3.86 to 8.24 days in female and tending to decrease with the increasing concentration, differed significantly with different concentrations ($P<0.05$)(Table – 12).

4.2. B.b. Effect of thuricide on post-embryonic development under topical method:

The larva of the untreated adults acquired considerably more pupation (89.43%) than that of the adults treated topically with any concentration of the thuricide ($P<0.05$). The pupation varied from 27.12 to 69.85 per cent among residue films of different concentrations and tending to decrease with the advancing concentration, the larval survival was affected by the residue film concentration significantly ($P<0.05$) (Table – 10).

In response to parent adults' treatment with residue films of different concentrations of thuricide the duration of the larval stage varied from 19.64 to 36.30 days and showed a tendency of increase with the increasing concentration in comparison to the untreated adults ($P<0.05$).

The pupa of the untreated adult gained hundred per cent emergence in comparison to that of the adults treated with any concentration of the thuricide through food ($P<0.05$). In response to treatment of adults with different concentrations of the thuricide, the emergene, varying from 29.76 to

Table -11

Net mortality in *D. obliqua* caused by "Thuricide" at different concentrations under different modes of treatment.

(Values are mean ± S.E.)

Mode of treatment	Concentration applied (%)	No. of larvae reared	No. of larvae died	No. of Pupae died	Total death	%net mortality
L.D.M.	0.05	60	24	12	36	52
	0.10	60	28	18	46	76
	0.50	60	34	19	53	86
	0.75	60	40	16	56	92
	1.00	60	45	12	57	94
T.M.	0.05	60	19	16	35	50
	0.10	60	27	22	49	78
	0.50	60	36	17	53	86
	0.75	60	42	14	56	92
	1.00	60	43	14	57	94
	Control	60	10	NIL	10	–

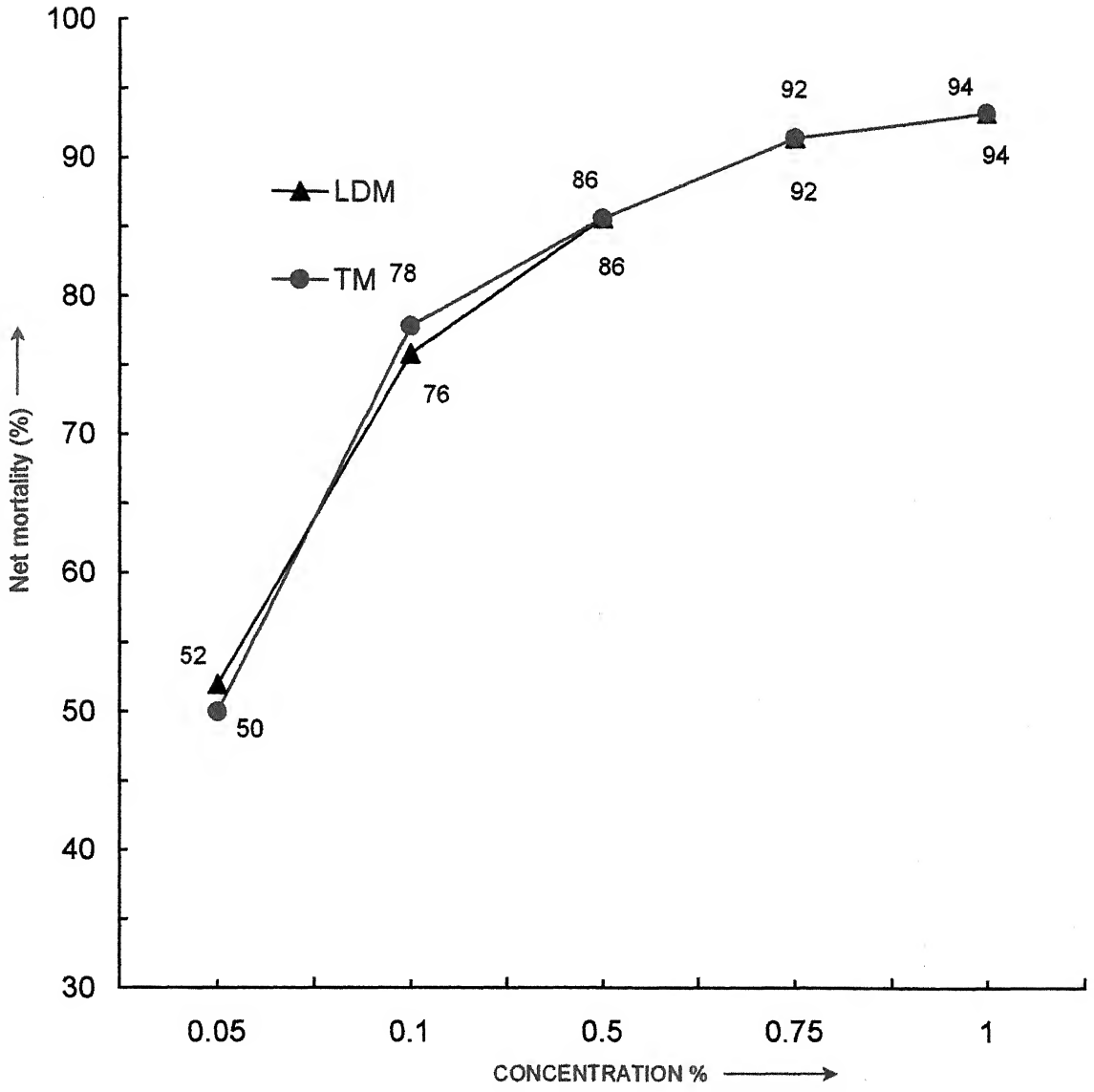


Figure 11. Net mortality in *D. obliqua* caused by "Thuricide" at different concentrations under LDM and TM modes of treatment.

Table – 12

Effect of "Thuricide" on longevity in male and female *D. obliqua* at different modes of treatment.

(Values are mean ± S.E.)

Mode of treatment	Concentration (%)	Longevity (In days)	
		Male	Female
L.D.M.	0.05	7.92±0.22	8.24±0.42
	0.10	7.86±0.44	7.84±0.44
	0.50	6.06±0.42	6.06±0.28
	0.75	5.04±0.22	5.50±0.20
	1.00	3.76±0.15	3.86±0.25
T.M.	0.05	8.60±0.22	8.90±0.42
	0.10	7.02±0.42	7.00±0.46
	0.50	6.06±0.45	6.87±0.37
	0.75	5.66±0.14	5.80±0.88
	1.00	3.68±0.32	3.94±0.36
	Control	11.35±0.22	13.76±0.24

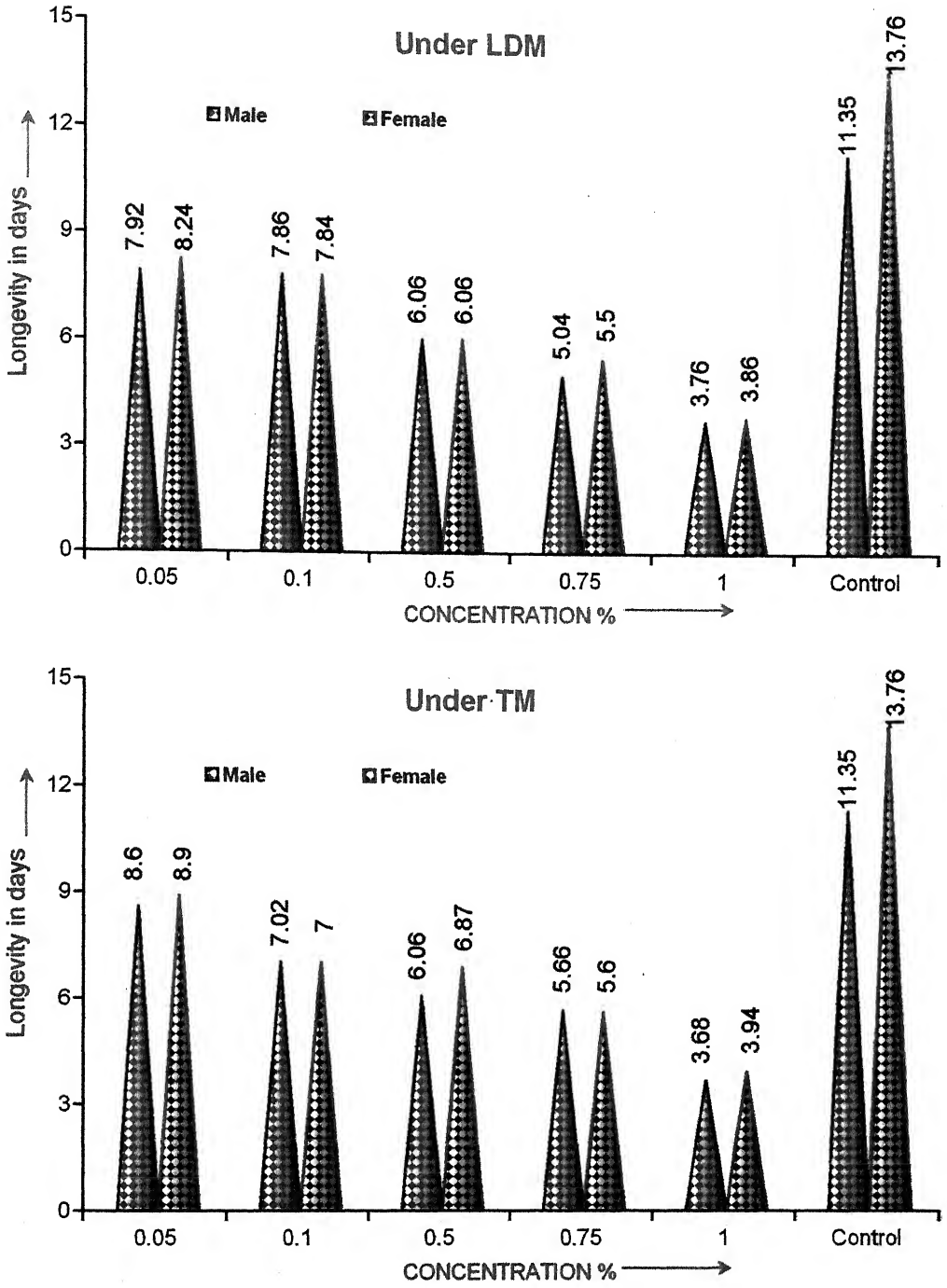


Figure 12. Effect of "Thuricide" on longevity in male and female *D. obliqua* at LDM and TM modes of treatment.

72.82 per cent and tending to be directly proportional to the concentration differed significantly from concentration to concentration ($P<0.05$).

The pupal period which varied from 14.94 to 28.70 days among residue film concentrations from 0.05 to 1.0 per cent and tended to be directly proportional to the strength of the residue film was found to be effective differently by the residue films of different concentrations of this microbial preparation ($P<0.05$) (Table – 10).

The net mortality, varying from 50 to 94 per cent showing direct proportionality to the concentration, differed significantly with the residue films of different concentrations of the thuricide ($P<0.05$) (Table – 11 ; Fig. -11).

Every concentration of the thuricide applied as residue film to the adult reduced the life-span of both male and female adults ($P<0.05$). As regards the influence of different concentrations of the thuricide as residue films on the longevity of adults, it varying from 3.68 to 8.60 days in male and 3.94 to 08.90 days in female and declining with the advancing concentration of thuricide, differed with the concentration of the residue film ($P<0.05$) (Table – 12 ; Fig.- 12).

4.2. C. Effect of Bactospeine on post embryonic development:

4.2. C.a. Effect of bactospeine on post-embryonic development under leaf dip method:

The untreated adults induced more larval survival as compared those treated by leaf dip method with any concentration of this microbial

preparation ($P<0.05$). In response to different concentrations of bactospeine under leaf dip method, the larval survival varying from 25.16 to 72.76 per cent and exhibiting a direct proportionality to the concentration, differed with different concentrations ($P<0.05$). Any concentration of this bacterial preparation applied through leaf dip method prolonged the larval period, varying from 20.86 to 33.35 days, as per statistical analysis, differed with concentrations ($P<0.05$), showing a tendency towards protraction with advancing concentration(Table – 13).

Leaf dip treatment with any concentration of this bacterial preparation curtailed the emergence considerably ($P<0.05$) as compared the untreated situation. In response to treatment by leaf dip method with different concentrations of bactospeine, the emergene, varying from 22.28 to 71.82 per cent and exhibiting a direct proportionality to the concentration, differed with the concentration significantly ($P<0.05$) (Table – 13 ; Fig. -14).

Further, any concentration of bactospeine prolonged the pupal period as compared with the control experiment ($P<0.05$). Tending to prolong with the increasing concentration, the pupal stage varied from 14.75 to 27.12 days among different concentrations and it was detected statistically to depend on the concentration ($P<0.05$) (Fig. –13).

The findings pertaining to the net mortality revealed that in response to leaf dip treatment with different concentrations of bactospeine, as per chi-square test, it varying from 34 to 84 per cent and increasing with the rise

Table - 13

Effect of "Bactospeine" on post embryonic development in *D. obliqua* at different concentrations under different modes of treatment.

(Values are mean ± S.E.)

Mode of treatment	Concentration (%)	Pupation (%)	Larval period (days)	Emergence (%)	Pupal period (days)
L.D.M.	0.05	72.76	20.86±0.12	71.82	14.75±0.12
	0.10	59.72	22.24±0.14	67.35	15.76±0.22
	0.50	48.46	26.18±0.44	54.36	18.52±0.24
	0.75	38.57	30.74±0.28	40.21	23.22±0.46
	1.00	25.16	33.35±0.79	22.28	27.12±0.22
T.M.	0.05	76.25	19.08±0.64	75.72	15.25±0.20
	0.10	60.72	22.02±1.14	70.25	16.60±0.24
	0.50	49.48	24.25±2.12	60.35	18.72±0.46
	0.75	38.84	28.42±0.12	42.11	22.15±0.48
	1.00	26.52	32.42±0.14	23.82	26.26±0.68
	Control	89.43	16.35±0.42	100.00	12.76±0.32

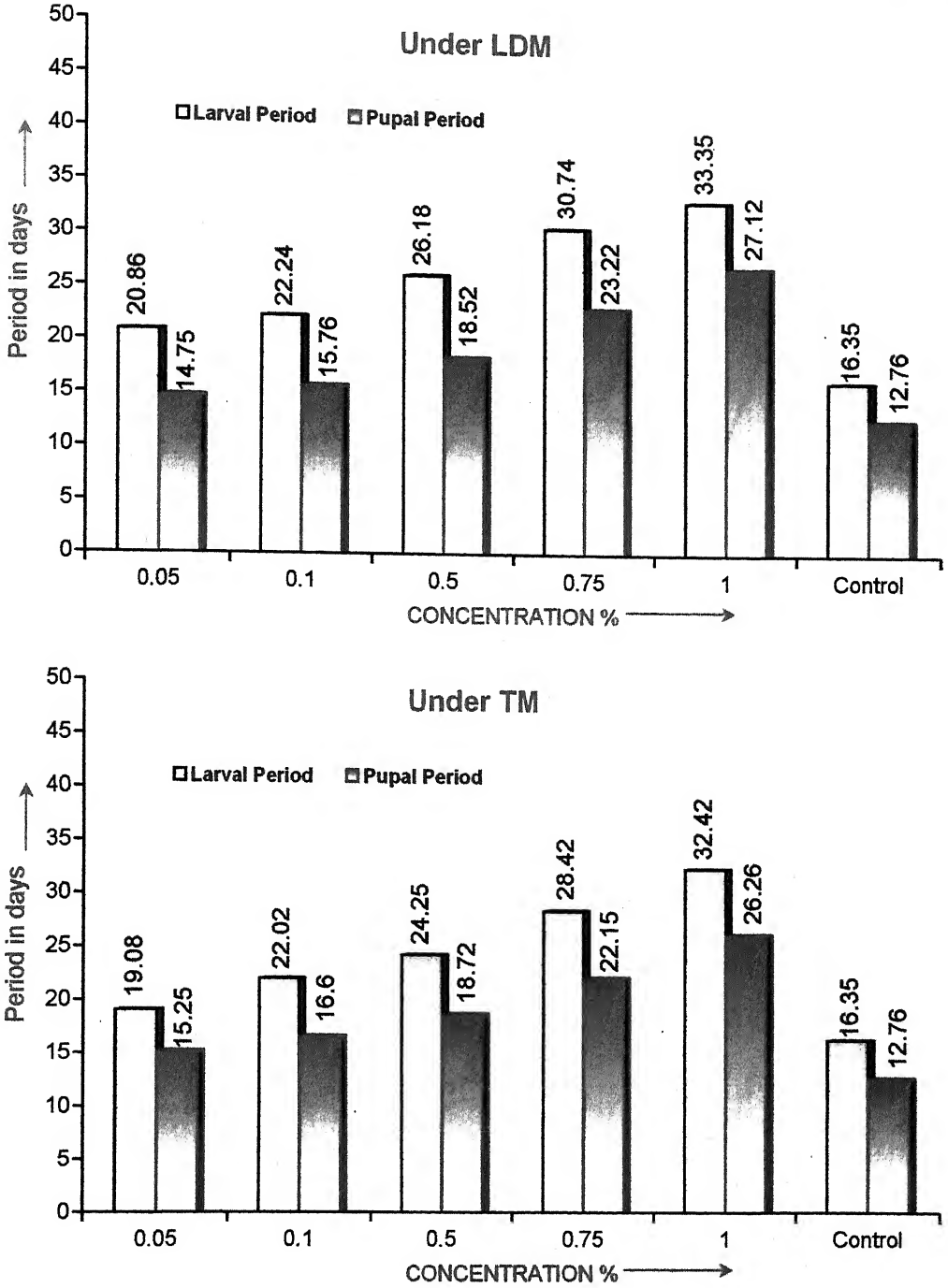
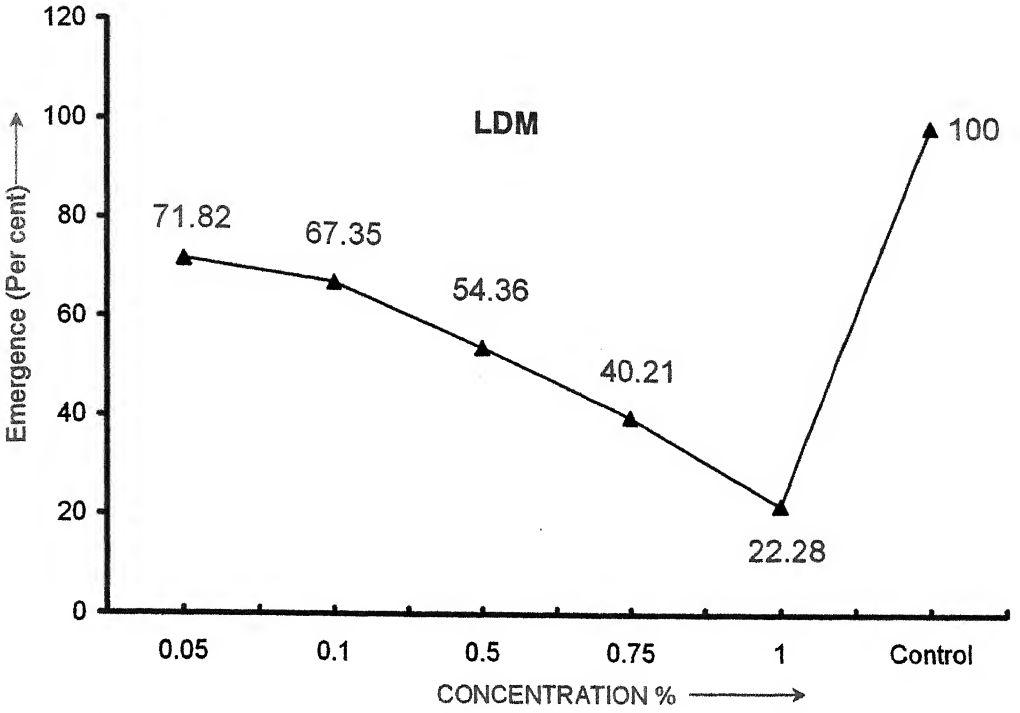


Figure 13. Effect of different concentrations of "Bactospeine" on larval and pupal period of *D. obliqua* under LDM and TM of treatment.



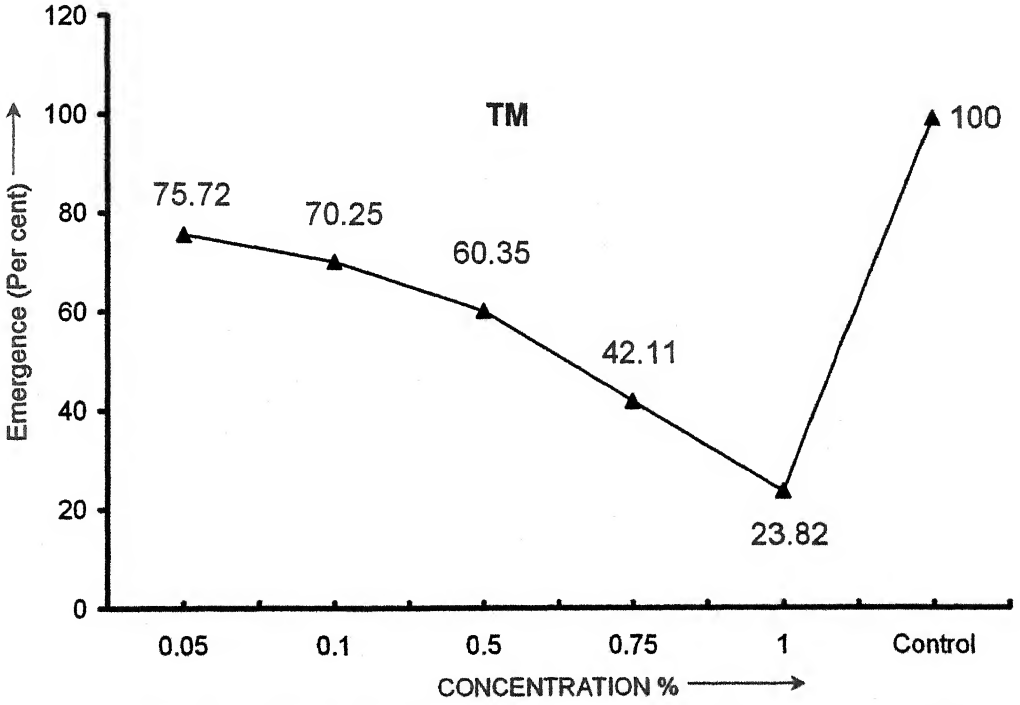


Figure 14. Effect of "Bactospeine" on emergence in *D. obliqua* at different concentrations under LDM and TM modes of treatment.

in the strength of bactospeine differed significantly with the concentration ($P<0.05$) (Table – 14 ; Fig. -15).

The leaf dip treatment with any concentration of the bactospeine reduced the life span of progeny adults of the both sexes as compared the untreated ones ($P<0.05$). In male moth, the longevity varies from 4.82 to 7.56 days and in female from 4.05 to 7.66 days differed with the concentration of bactospeine significantly ($P<0.05$) (Table – 15).

4.2. C.b. Effect of bactospeine on post-embryonic development under topical method:

Any concentration of the bactospeine applied earlier under topical method to adults, reduced the larval survival and delayed the pupation as compared the untreated condition of parents ($P<0.05$). The larval survival, varying from 26.52 to 76.25 per cent among different concentrations from 0.05 to 1.0 per cent and appearing to be indirectly proportional to them, was affected differently by different concentrations of bactospeine ($P<0.05$). Further, the larval period varying from 19.08 to 32.42 days and prolonging with the advancing concentration of bactospeine, differed from concentration to concentration applied earlier by topical method ($P<0.05$) (Table – 13).

Under topical method, the emergence was curtailed significantly by any concentration of this pest controlling agent ($P<0.05$). In the concentration range of 0.05 to 1.0 per cent, the emregence, varying from 23.82 to 75.72 per cent and tending to decrease with the increasing concentration, differed from

Table -14

Net mortality in *D. obliqua* caused by "Bactospeine" at different concentrations under different modes of treatment.
(Values are mean ± S.E.)

Mode of treatment	Concentration applied (%)	No. of larvae reared	No. of larvae died	No. of Pupae died	Total death	%net mortality
L.D.M.	0.05	60	10	17	27	34
	0.10	60	16	17	33	46
	0.50	60	22	16	38	56
	0.75	60	30	18	48	76
	1.00	60	35	17	52	84
T.M.	0.05	60	11	16	27	34
	0.10	60	15	18	33	46
	0.50	60	19	19	38	56
	0.75	60	22	22	44	68
	1.00	60	38	14	52	84
	Control	60	10	NIL	10	–

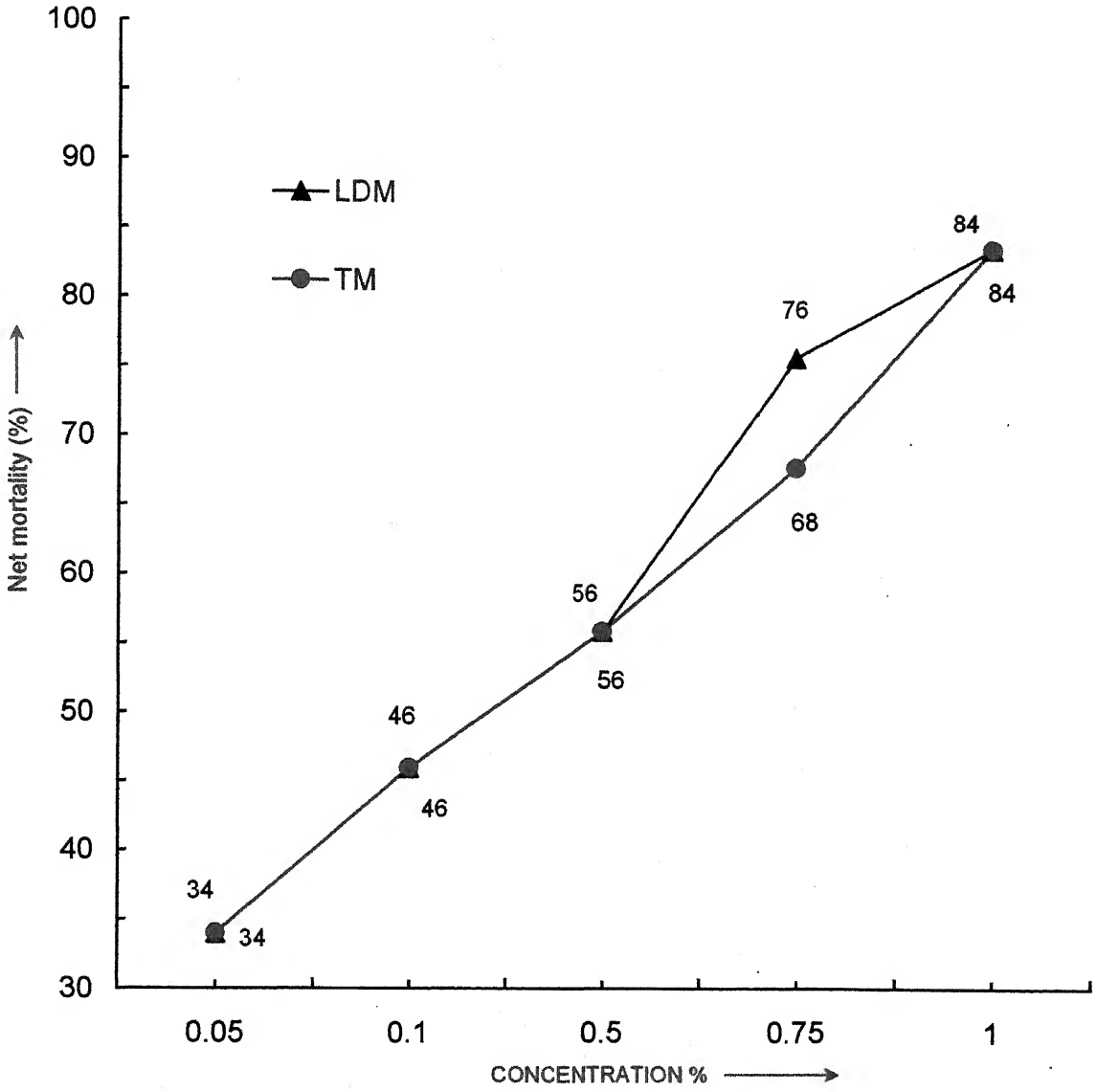


Figure 15. Net mortality in *D. obliqua* caused by "Bactospeine" at different concentrations under LDM and TM modes of treatment.

Table - 15

Effect of "Bactospeine" on longevity in male and female *D. obliqua* at different modes of treatment.

(Values are mean ± S.E.)

Mode of treatment	Concentration (%)	Longevity (In days)	
		Male	Female
L.D.M.	0.05	7.56±0.12	7.66±0.14
	0.10	6.80±0.14	7.20±0.13
	0.50	6.42±0.43	6.09±0.82
	0.75	6.02±0.03	6.80±0.60
	1.00	4.82±0.16	4.05±0.26
T.M.	0.05	7.52±0.12	7.90±0.24
	0.10	7.82±0.14	6.85±0.58
	0.50	6.48±0.14	6.60±0.36
	0.75	6.26±0.18	5.92±0.48
	1.00	4.96±0.26	3.36±0.32
	Control	11.35±0.22	13.76±0.24

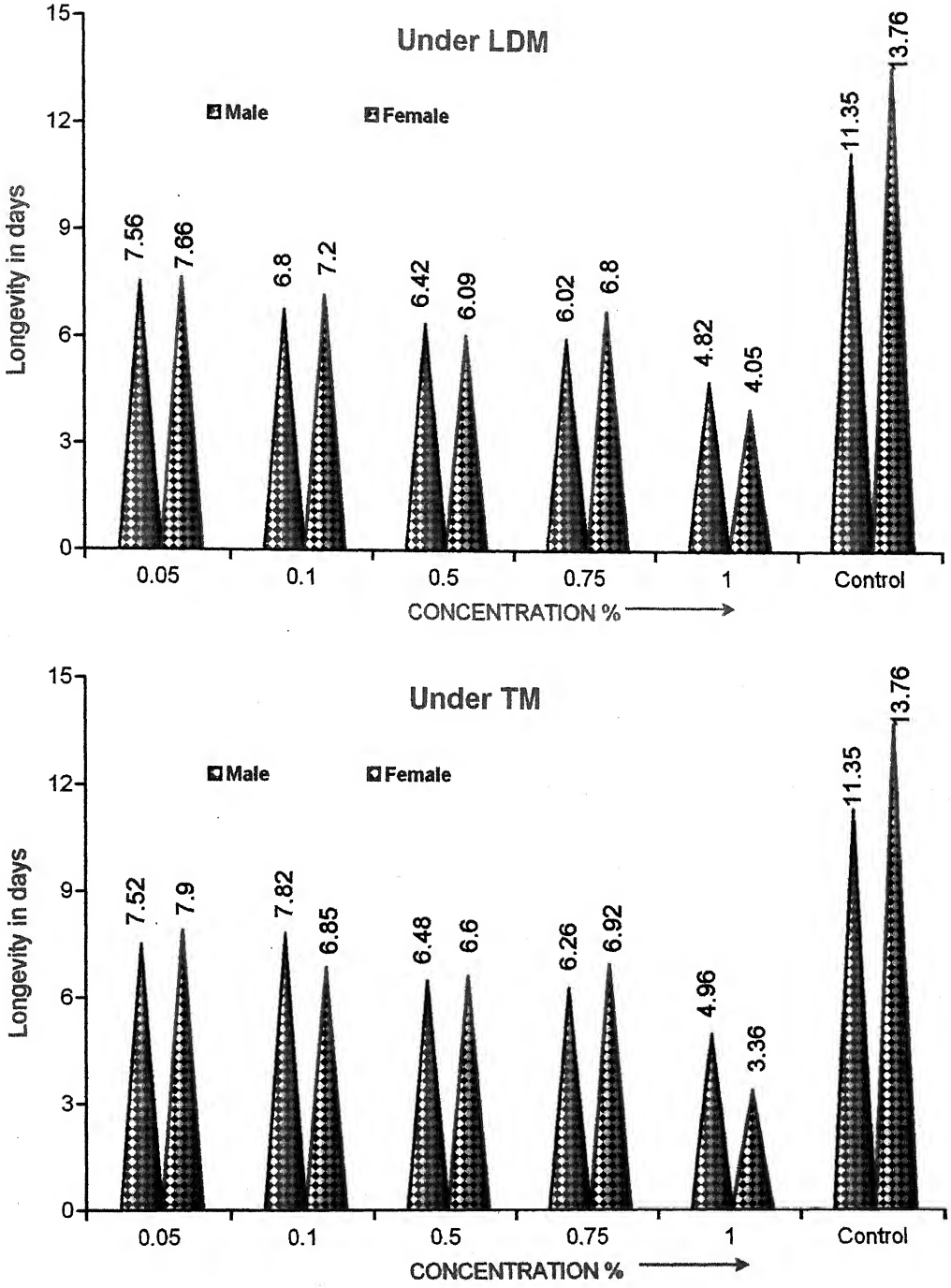


Figure 16. Effect of "Bactospeine" on longevity in male and female *D. obliqua* at LDM and TM modes of treatment.

concentration to concentration significantly and likewise, the pupal period, varying from 15.25 to 26.26 days among concentrations from .05 to 1.0 per cent also depended significantly ($P<0.05$) on these concentrations but it exhibited the direct proportionality to the concentration of the bactospeine under topical method of treatment (Table -13).

The net mortality under the residue film treatment varied from 34.00 to 84.00 per cent, increasing with the advancing concentration and Chi square test detected it to depend on the concentration of bactospeine significantly ($P<0.05$) (Table – 14).

The male adult obtained from the control experiment had more life span (11.35 days) as compared to the adult obtained from the parents treated with any concentration of bactospeine ($P<0.05$) and this fact was applicable to the female adult also ($P<0.05$). The longevity of the male adult, varying from 4.96 to 7.52 days and that of the female adult, varying from 3.36 to 7.90 days, tended to decrease with the increase in the concentration of the bactospeine. However, the statistical analysis revealed that concentration of bactospeine inducing longevity in both sexes significantly ($P<0.05$) (Table – 15).

Depending on their concentration, the used bacterial preparations cause net mortality, ranging from about 34 per cent to 96 per cent and this mortality mostly increases with the increase in the concentration. The administration of dipel by leaf dip method causes more larval mortality than its application through the topical method. As regards comparative effectiveness of

the tested bacterial preparations, the results permit us to arrange them as dipel, thuricide and bactospeine in descending order.

It is evident from the results of the experiments that lethal concentration of dipel had marked effect on the larval development. In this context, it would be worthy to mention that surviving larvae of *D. obliqua* were significantly shorter in length and lighter in weight when compared with control. Larvae also manifested prolongation in larval duration besides pupation of *D. obliqua* larvae. Over and above this, more larval casuality was also noted in treated moths.

There are number of reports on the fate of larvae which survived to the treatment of *B. thuringiensis*, but the test insects employed by them were altogether different. Schmidth (1979), Cantwell *et.al.* (1986) and Chandra *et.al.* (1999) reported the prolongation of larval duration by 3 to 4 weeks. Matter and Zohdy (1981) Chaturvedi (2003) and Bajpai (2003) also recorded a similar trend in larval development of *H. armigera, U.pulchella* and *L. orbonalis* respectively. The present findings were also found to corroborate with the results of the earlier workers.

In the present investigation, it was observed that the pupa of *D. obliqua*, developed from the bacterial preparation treated larvae, took more time to reach the adult stage. This finding in full alignment with the report of earlier workers. Considering the emergence of adults from the treated stock, the present author has noticed the poor emergence of *D. obliqua*. A similar view was also

held by Yang *et. al.* (1985) and Hernadez *et al.* (2005) while studying this aspect in *S. litura*.

In the present study the moths which emerged from the treated larvae were significantly smaller in size and oviposited less in comparison to moths emerged from the untreated larvae. Further, it was observed that the life span of such moths was remarkably shorter than those emerging from the healthy untreated larvae.

A detailed study of literature divulge the drifting opinions regarding this aspect. Yang *et. al.* (1985) and Hernadez *et al.* (2005) recorded the total loss of oviposition in *S. litura* adults emerging from the *Bacillus* treated larvae. However, Khalique et. *al.* (1982) observed that the treatment of *H. armigera* larvae with 10 to 20 g. of spore - δ- endotoxin complex of *B.t.* – 145 resulted in the reduction of fecundity and longevity of the moth. Chandra *et. al* (1999); Navrozidis *et. al* (2000) and Nault *et. al.* (2000) also reported same findings in *H.* armigera, *B. oleae* and colorado potato beetle respectively. Devaki and Krishnayya (2004) and Naglaa *et al.* (2004) reported same opinion in *S. litura* and *Galleria mellonella* respectively. The present findings are in agreement with the findings of these workers. Contrary to the findings of earlier workers, Salama and Zaki (1986) demonstrated that there is no effect on longevity and fecundity of *S. littoralis* when heavy concentration of dipel (5.0%) was applied to the prepupal stage of the insect. This variation may be due to the high concentration of the pathogen and different stages of the insect subjected to the treatment.

As regards the viability of eggs, it is obvious from the results that there was significant effect on hatching of eggs laid by the moths emerged from treated stock in comparison with untreated moths. Contrary to the findings of this investigation, Dubois *et. al.* (1988) reported no significant effect on hatching of eggs in case of Gypsy moth (*L. dispar).*

On the basis of overall development of treated larvae, it can be inferred that the larvae of *D. obliqua*, after intoxication with lethal concentration of bacterial preparations elicited detrimental effect on the biology of experimental insect.

It is widely accepted that insects exposed to crops treated with *B. thuringiensis* for different duration had differential response to their survival and rate of mortality. Dulmage *et. al.* (1978) reported that larvae of *H. virescens* exposed for longer duration to *B. thuringiensis* exhibited higher susceptibility and less recovery from infection, whereas the larvae exposed for short period demonstrated complete recovery from the infection of pathogen. Salama *et. al.* (1981) observed that larval exposure for one day to high concentration of *B. thuringiensis* led to hundred per cent mortality in *H. armigera* after 21 days, while the larval exposure for 2-3 days resulted the same mortality (100%) within 14 days. Fast and Regnier (1984) also reported that extension of larval exposure of spruce bud worm to *B. thuringiensis* from 1 day to 6 days resulted in 500 fold reduction in LC_{50} value. In the present investigation the effect of pathogen shows positive co-relation with the mortality. Ruperez (1966), Larson and Ignoffo (1971)

Chen *et. al.* (1974); West *et. al.* (1997); Chandra *et. al.* (1999); Siegfried *et. al.* (2000); Chaturvedi (2003); Bajpai (2003); Fetoh and Azazy (2004); Naglaa *et al.* (2004); Kumar and Gujar (2005) and Gopalkrishnan and Gangavisalakshy (2005) also reported same results in different insects. Thus present results are in consonance to the findings of earlier workers.

4.3. EFFECT OF BACTERIAL PREPARATION ON REPRODUCTION:

4.3. A. Effect of dipel on reproduction:

4.3. A.a. Effect of dipel on reproduction under leaf dip mehtod:

Any concentration of the dipel applied by leaf dip method, increased the pre-oviposition period ($P<0.05$). Under leaf dip treatment, this period varied from 3.03 to 3.86 days among different concentrations of the dipel showing indirect proportionality to the concentration ($P<0.05$). Under this method of treatment, every concentration of the dipel affected the oviposition period ($P<0.05$). As regards the influence of different concentrations of this bacterial preparation on the duration of egg laying, the oviposition period, varying from 2.12 to 5.22 days and decreasing with the falling concentration, differed significantly with different concentrations of the dipel ($P<0.05$) (Table – 16).

Further, the treatment by leaf dip method with any strength of the dipel reduced the fecundity significantly ($P<0.01$). The fecundity in response to treatment with different strengths of this micorbial preparation varied from 40.4 to

Table -16

Effect of "Dipel" on reproductive periods in *D. obliqua*.
(Values are mean ± S.E.)

Mode of treatment	Concentration (%)	Pre-oviposition period (days)	Oviposition period (days)
L.D.M.	0.05	3.03±0.26	5.22±0.33
	0.10	3.46±0.26	5.00±0.50
	0.50	3.06±0.48	4.32±0.42
	0.75	3.85±0.12	3.06±0.18
	1.00	3.86±0.14	2.12±0.12
T.M.	0.05	3.02±0.44	5.62±0.12
	0.10	3.25±0.32	5.00±0.24
	0.50	3.85±0.41	4.41±0.18
	0.75	3.82±0.14	3.34±0.44
	1.00	3.82±0.12	2.24±0.43
	Control	1.68±0.25	4.82±0.76

Table - 17

Effect of "Dipel" on fecundity and fertility in *D. obliqua*.
(Values are mean ± S.E.)

Mode of treatment	Concentrations (%)	No. of eggs laid by a female	No. of eggs hatched	Hatched (%)	Incubation period (days)
L.D.M.	0.05	120.6±3.42	69.8±3.62	50.4	3.24±0.64
	0.10	126.4±3.58	63.5±4.24	50.3	3.56±0.42
	0.50	110.3±2.69	44.3±2.78	40.2	3.92±0.12
	0.75	81.2±3.76	23.2±4.32	28.6	3.94±0.22
	1.00	40.4±2.22	4.3±1.44	8.3	5.42±0.13
T.M.	0.05	130.4±2.42	74.5±2.24	54.6	3.26±0.52
	0.10	122.5±3.30	65.4±2.32	53.4	3.44±0.31
	0.50	111.2±2.45	48.3±2.35	43.5	3.87±0.23
	0.75	89.4±3.28	19.1±3.42	21.4	3.97±0.13
	1.00	50.3±2.46	7.7±1.72	14.6	5.02±0.12
	Control	346.2±4..24	310.3±2.14	89.6	2.78±0.24

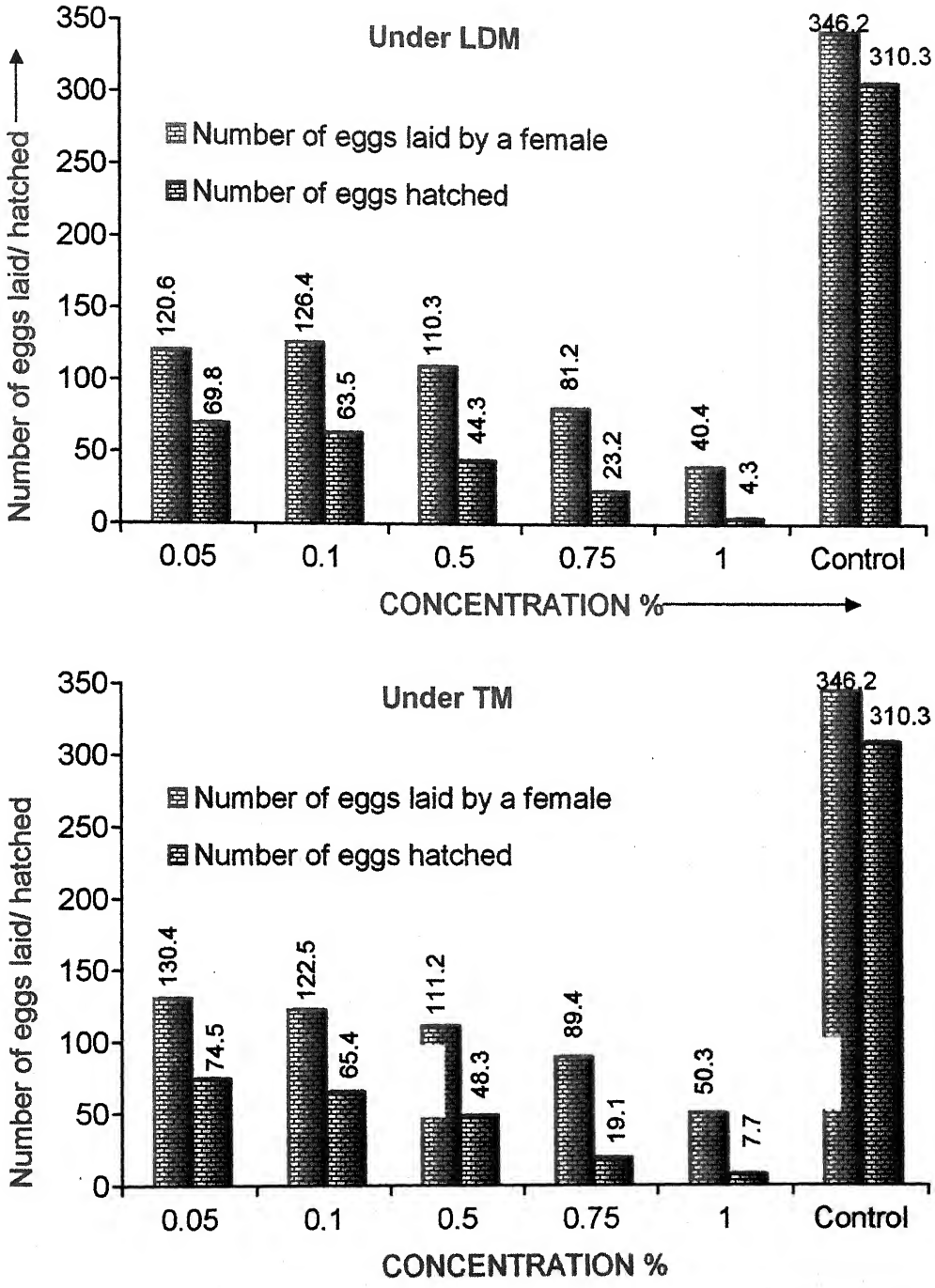


Figure 17. Effect of "Dipel" on fecundity and fertility in *D. obliqua* under LDM and TM modes of treatment.

120.6 eggs/female and the analysis of variance revealed that 0.05 and 0.10 per cent affected the fecundity identically and that among concentrations from 0.50 to 1.00 per cent, the fecundity differed with the different concentrations, showing indirect proportionality to the concentration of the dipel (Table – 17 ; Fig. -17).

The adult's treatment with any concentration by leaf dip method reduced the fertility of the *D. obliqua*. The percentage of eggs hatched per female reduced significantly ($P<0.05$). In response to different concentrations of the dipel applied through leaf dip method, the fertility varying from 8.3 to 50.4 per cent and decreasing with the increasing strength of bio pesticide depended on the concentration of the dipel ($P<0.05$) (Table – 17 ; Fig. -17).

Every concentration of the dipel applied by leaf dip method, prolongs the incubation period ($P<0.05$). The duration of the egg stage, varying from 3.24 to 5.42 days, differed with the concentrations of dipel ($P<0.05$). The concentrations 0.05, 0.10, 0.50. and 0.75 per cent affected the incubation period identically (3.24 to 3.94 days) causing less delay in the incubation period as compared to 1.0 per cent concentration, that cause more delay i.e. 5.40 days.

The reduction in the fecundity of the moth under the leaf dip treatment, varied from 39.2 to 79.0 per cent among different concentrations of the dipel and exhibiting indirect proportionality to the concentration, was affected differently by different concentrations ($P<0.05$) (Table – 18).

Further, the net sterility which varied from 7.75 to 86.38 per cent among different concentrations under leaf dip treatment differed significantly from

concentration to concentration and it also increases with increase in the concentration (Table – 18). Exactly in the same way, the per cent control over the reproduction under the influence of different concentrations of the dipel, varying from 50.4 to 93.6 per cent and increasing with the advancing concentration depended on the strength of the dipel applied ($P<0.05$).

4.3. A.b. Effect of dipel on reproduction under topical method:

The pre-oviposition period was affected by every concentration of the dipel under topical method ($P<0.05$). In response to adult's treatment under topical method with different concentrations of the dipel, the pre-oviposition period (3.03 to 3.86 days) was affected identically by 0.05 and 0.10 per cent concentrations ($P<0.05$) and like wise, it was also more effective by 0.50 , 0.75 and 1.0 per cent concentrations, cause more prolongation.

As regards the influence of the dipel under topical method on oviposition period, every concentration of the dipel caused change in the oviposition period. This period varied from 2.12 to 5.22 days and appeared to decrease with the increasing concentration, differed significantly with the residue film concentrations ($P<0.05$) (Table – 16).

The fecundity (eggs/female) was reduced under topical method with any concentration of the dipel applied to the adult ($P<0.05$). The fecundity varied from 50.3 to 130.4 eggs/female in response to treatment with residue films of different concentrations and appearing to decrease with the advancing concentrations of dipel and tending to be indirectly proportional to the

Table - 18

Per cent reduction in fecundity, per cent net sterility and per cent control over reproduction in *D. obliqua* caused by "Dipel" under different modes of treatment.
(Values are mean ± S.E.)

Mode of treatment	Concentrations (%)	(%) Reduction in fecundity	(%) Net sterility	(%) Control over reproduction
I.D.M.	0.05	39.2	7.75	50.4
	0.10	45.0	35.62	56.8
	0.50	62.3	40.34	78.9
	0.75	70.7	56.72	90.6
	1.00	79.0	86.38	94.6
T.M.	0.05	38.6	10.62	47.2
	0.10	44.4	40.37	54.4
	0.50	56.3	52.72	73.9
	0.75	68.4	58.64	88.9
	1.00	72.2	80.40	94.0

concentration differed significantly with the concentrations. Every concentration (0.05 to 1.0%) caused reduction in fecundity significantly ($P<0.05$) (Table – 17)

Further, every concentration of the dipel applied by topical method lead to reduction in hatching of eggs. In response to topical treatment of adults with dipel, the hatchability of eggs, varying from 14.6 to 54.6 per cent among different concentrations and tending indirectly proportional to the concentrations was affected differently by different concentrations as residue film ($P<0.05$). The egg stage, incubation period, was also affected significantly by every concentration of the dipel applied to adults under topical mehtod ($P<0.05$). The incubation period, varying from 3.26 to 5.02 days among different concentrations and increasing with the increasing concentration of dipel ($P<0.05$) (Table – 17).

Every concentration of the dipel applied by topical method to adults caused reduction in the fecundity which, varying from 38.6 to 72.2 per cent among the different concentrations of this microbial preparation and exhibiting direct relationship to the concentration, was detected by chi-square to differ from concentration to concentration of the dipel applied earlier to adults ($P<0.05$). Further, in response to treatment of female with different concentrations of the dipel, the per cent net sterility and per cent control over reproduction, varying from 10.62 to 80.40 per cent and 47.2 to 94.0 per cent respectively and increasing with the increasing concentrations, was affected differently by the concentrations of dipel ($P<0.05$) (Table -18).

4.3. B. Effect of thuricide on reproduction:

4.3. B.a. Effect of thuricide on reproduction under leaf dip method:

The treatment by leaf dip method with every concentration of the thuricide delayed the sexual maturity ($P<0.05$). The pre-oviposition period of the treated adults varied from 2.98 to 3.64 days in response to different concentrations of the thuricide and appeared to be directly proportional to the concentrations applied ($P<0.05$). All of these concentrations also affected the oviposition period which varied from 2.28 to 5.36 days among different concentrations of the thuricide applied by leaf dip method and it decreased with the increasing concentration. The analysis of variance test revealed that under leaf dip method of treatment, the oviposition period depended on the concentration of the thuricide ($P<0.05$) exhibiting increase in the egg stage with increasing concentration of thuricide (Table -19).

The female's treatment with all the concentrations of thuricide by leaf dip method reduced its fecundity precipitiously ($P<0.05$). In response to female's treatment by leaf dip method with different concentrations of thuricide, the number of eggs laid by a female, varying 57.4 to 130.3 eggs and exhibiting indirect proportionality to the concentration, was affected differently by these concentrations ($P<0.05$). Further, the female treated by leaf dip method with different concentrations of the thuricide caused less fertility as compared the

Table -19

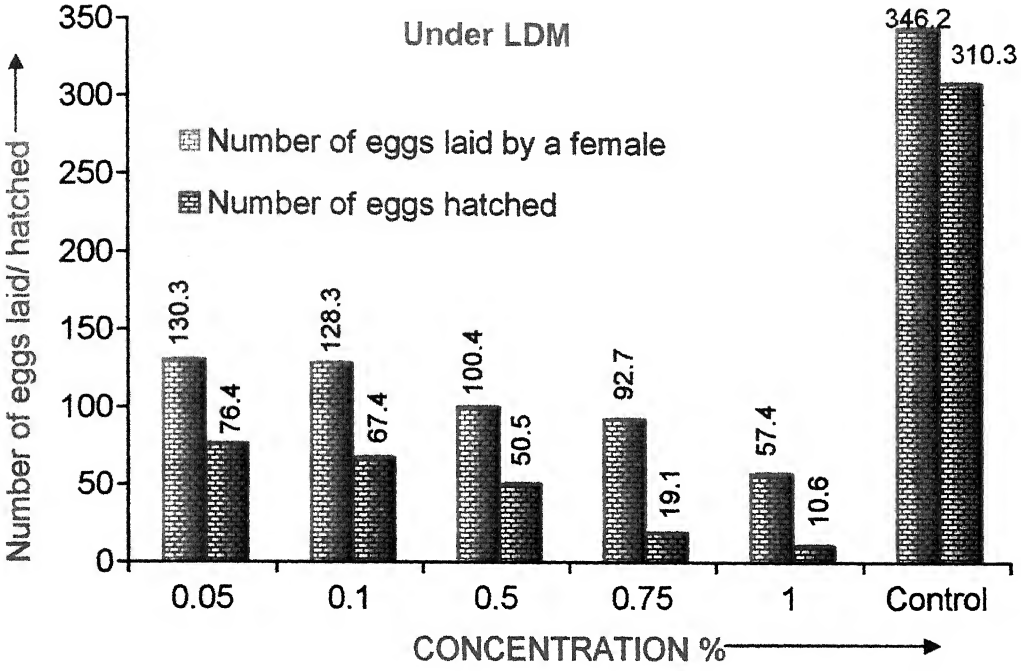
Effect of "Thuricide" on reproductive periods in *D. obliqua*.
(Values are mean ± S.E.)

Mode of treatment	Concentration (%)	Pre-oviposition period (days)	Oviposition period (days)
L.D.M.	0.05	2.98±0.44	5.36±0.34
	0.10	3.06±0.16	5.84±0.25
	0.50	3.83±0.18	4.60±0.14
	0.75	3.38±0.42	3.21±0.13
	1.00	3.64±0.24	2.28±0.22
T.M.	0.05	3.26±0.15	5.45±0.38
	0.10	3.26±0.16	5.21±0.12
	0.50	3.34±0.18	4.71±0.22
	0.75	3.36±0.14	3.75±0.12
	1.00	3.69±0.12	2.54±0.14
	Control	1.68±0.25	4.82±0.76

Table – 20

Effect of "Thuricide" on fecundity and fertility in *D. obliqua*.
(Values are mean ± S.E.)

Mode of treatment	Concentrations (%)	No.of eggs laid by a female	No. of eggs hatched	Hatched (%)	Incubation period (days)
L.D.M.	0.05	130.3±2.24	76.4±2.84	58.4	3.44±0.72
	0.10	128.3±3.63	67.4±3.4.6	52.6	3.54±0.34
	0.50	100.4±4.74	50.5±3.72	50.3	3.62±0.28
	0.75	92.7±2.52	19.1±3.10	20.7	3.85±0.17
	1.00	57.4±3.12	10.6±3.24	18.3	4.64±0.12
T.M.	0.05	137.5±2.34	79.9±3.44	58.3	3.35±0.64
	0.10	130.4±2.48	69.7±3.52	53.5	3.52±0.38
	0.50	101.7±2.32	51.2±2.42	50.4	3.83±0.22
	0.75	97.9±2.78	23.1±3.14	23.7	3.87±0.18
	1.00	64.7±3.44	13.1±2.74	20.4	4.56±0.22
	Control	346.2±4.24	310.3±2.14	89.6	2.78±0.24



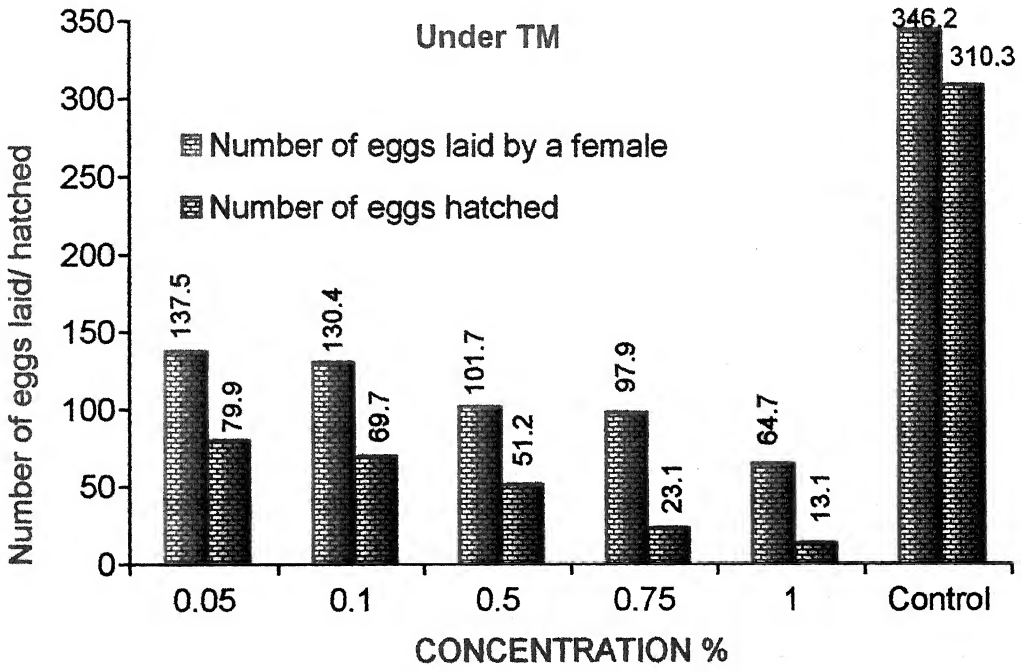


Figure 18. Effect of "Thuricide" on fecundity and fertility in *D. obliqua* under LDM and TM modes of treatment.

untreated female ($P<0.05$). The percentage of eggs hatched, varied from 18.3 to 58.4 per cent among different concentrations, exhibiting decrease in hatching with the increase in concentration and it differed significantly with the concentrations ($P<0.05$). Further more, the treatment of the female with any concentration of thuricide by leaf dip method prolonged the incubation period ($P<0.05$) which varied from 3.42 to 4.62 days in response to different concentrations and depended significantly on the concentration ($P<0.05$), exhibiting increase in the egg stage with increasing concentrations of thuricide. (Table- 20 ; Fig. - 18).

The female's treatment by leaf dip method with any concentration of thuricide caused reduction in fecundity, net sterility and control over the reproduction. The reduction in fecundity varied from 36.2 to 74.5 per cent. Like wise, the net sterility also varies from 6.78 to 72.35 per cent and also control over the reproduction, varying from 44.5 to 94.6 per cent. All these observations exhibit direct proportionality to the concentrations of thuricide, differed significantly from concentration to concentration ($P<0.05$)(Table – 21).

4.3. B.b. Effect of thuricide on reproduction under topical method:

The female treated with any concentration of the thuricide under topical method increase the pre-oviposition period than that of the untreated females ($P<0.05$). In response to topical treatment of adults with different concentrations of this microbial preparation, the pre-oviposition period varied

from 3.26 to 3.69 days, was affected significantly by these concentrations ($P<0.05$). Likewise, every concentration of thuricide affected oviposition period significantly ($P<0.05$). In response to female's treatment by topical method with different concentrations of the thuricide, the duration of egg laying, varying from 2.54 to 5.45 days and exhibiting direct relationship to the concentration, differed significantly with applied strengths ($P<0.05$)(Table – 19).

The female's treatment by topical method with different concentrations of the thuricide caused reduction in fecundity ($P<0.05$). In response to female's treatment under topical method with different concentrations of thuricide, the number of eggs laid by a female, varying from 64.7 to 137.5 eggs and exhibiting indirect proportionality to the concentration, was affected differently by these concentrations ($P<0.05$). As regards the effect of topical treatment with different concentrations of thuricide on the fertility i.e., the per-cent eggs hatched/female, varying from 20.5 to 58.4 and decreasing with the increasing concentrations, differed significantly with the residue films of different concentrations of the thuricide ($P<0.05$) (Table – 20 ; Fig. -18).

As regards the influence of different concentrations of the thuricide applied under topical method to adults on the incubation period, the duration of egg stage varied from 3.35 to 4.56 days among different concentrations and appeared to increase with increasing concentrations of thuricide. The statistical analysis revealed that the different concentrations affected incubation period significantly ($P<0.05$) (Table – 20).

Table - 21

Per cent reduction in fecundity, per cent net sterility and per cent control over reproduction in *D. obliqua* caused by "Thuricide" under different modes of treatment
(Values are mean ± S.E.)

Mode of treatment	Concentrations (%)	(%) Reduction in fecundity	(%) Net sterility	(%) Control over reproduction
L.D.M.	0.05	36.2	6.78	44.5
	0.10	42.7	34.36	53.6
	0.50	56.5	36.42	73.2
	0.75	67.4	51.36	85.2
	1.00	74.5	72.35	94.4
T.M.	0.05	37.3	5.93	45.4
	0.10	42.1	34.42	52.5
	0.50	59.0	51.76	74.2
	0.75	66.0	62.43	83.4
	1.00	72.7	72.48	93.6

Further, in response to topical treatment of adults with different strengths of the thuricide, the reduction in fecundity, net sterility and control over reproduction, varying from 37.3 to 72.7, from 5.93 to 72.48 and from 45.4 to 93.6 per cent respectively and showing direct proportionality to the concentration, differed significantly with different concentrations of thuricide (Table – 21).

4.3. C. Effect of bactospeine on reproduciton:

4.3. C.a. Effect of bactospeine on reproduction under leaf dip method:

The treatment of the female with any concentration of the bactospeine prolonged the pre-oviposition period remarkably ($P<0.05$) and under such method of treatment with different concentrations of this microbial preparation, the pre-oviposition period varied from 2.64 to 3.64 days and tended to be directly proportional with the concentration. Bactospeine exerted prolonging influence significantly ($P<0.05$) (Table – 22).

The female's treatment with any concentration of the bactospeine under leaf dip method also affected the oviposition period significantly ($P<0.05$) and in response to female's treatment with different strengths of the bactospeine, the oviposition period varying from 2.38 to 5.38 days and tending to be indirectly proportional to the concentration differed from concentration to concentration significantly ($P<0.05$)(Table - 22).

Table - 22

Effect of "Bactospeine" on reproductive periods in *D. obliqua*.
(Values are mean ± S.E.)

Mode of treatment	Concentration (%)	Pre-oviposition period (days)	Oviposition period (days)
L.D.M.	0.05	2.64±0.24	5.38±0.22
	0.10	2.79±0.14	5.24±0.24
	0.50	2.86±0.12	4.48±0.14
	0.75	2.98±0.16	3.00±0.18
	1.00	3.64±0.26	2.38±0.25
T.M.	0.05	2.64±0.14	5.46±0.26
	0.10	2.58±0.16	5.50±0.22
	0.50	2.76±0.18	4.64±0.26
	0.75	2.84±0.12	3.31±0.22
	1.00	3.62±0.12	2.65±0.25
	Control	1.68±0.25	4.82±0.76

Table - 23

Effect of "Bactospeine" on fecundity and fertility in *D. obliqua*.
(Values are mean ± S.E.)

Mode of treatment	Concentrations (%)	No.of eggs laid by a female	No. of eggs hatched	Hatched (%)	Incubation period (days)
L.D.M.	0.05	131.4±3.16	80.6±2.86	61.5	3.16±0.26
	0.10	128.4±2.26	67.0±1.46	52.2	3.12±0.48
	0.50	100.3±3.38	50.1±3.44	50.0	3.23±0.13
	0.75	90.6±6.38	31.1±1.78	34.4	3.43±0.35
	1.00	60.5±3.34	12.8±2.54	21.3	4.85±0.24
T.M.	0.05	132.6±3.36	83.6±2.81	62.7	3.16±0.43
	0.10	140.7±3.23	74.8±2.42	53.2	3.17±0.18
	0.50	100.6±5.30	51.7±2.37	51.4	3.18±0.13
	0.75	92.8±2.42	35.4±2.42	38.2	3.82±0.32
	1.00	77.8±3.12	18.2±2.49	23.3	4.82±0.45
	Control	346.4±4.28	310.3±2.14	89.6	2.78±0.24

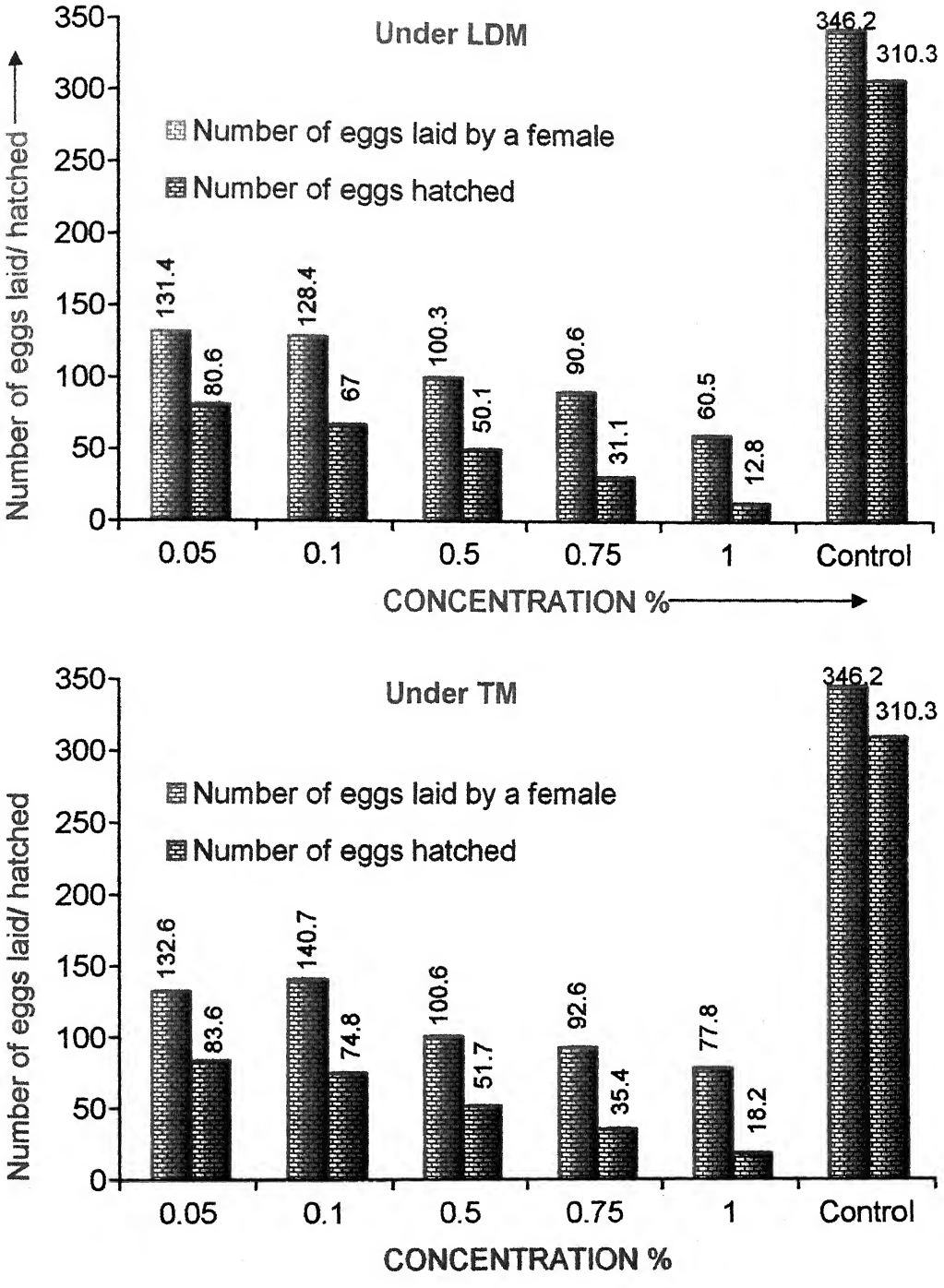


Figure 19. Effect of "Bactospeine" on fecundity and fertility in *D. obliqua* under LDM and TM modes of treatment.

Further, every concentration of bactospeine applied by leaf dip method caused considerable reduction in the fecundity as compared to the fecundity of the untreated females ($P<0.05$). In this context the results revealed that the different concentrations of this bacterial preparation caused a female to lay from 60.5 to 131.5 eggs which depended statistically on the strength of bactospeine ($P<0.05$) and decreased with the advancing concentration. The leaf dip treatment with any concentration reduced the percentage of hatching of eggs also. In response to different concentrations of the bactospeine applied by leaf dip method, hatching of eggs varies from 21.3 to 61.5 per cent, decreasing with the increasing strength depended on the concentration of the bactospeine ($P<0.05$). The influence of different concentrations of the bactospeine under leaf dip treatment, on the incubation period was affected differently ($P<0.05$). The incubation period varies from 3.16 to 4.85 days, prolongs with advance concentrations of bactospeine (Table 23 ; Fig. -19).

The leaf dip administration of different concentrations of bactospeine induced 27.5 to 68.4 per cent reduction in the fecundity which differing significantly with the concentration of this bacterial preparation ($P<0.05$). The reduction in the fecundity increased with the increasing concentrations of bactospeine. In the same way, the net sterility, varies from 11.46 to 56.54 per cent among females treated by leaf dip method with different concentrations. It was also observed that the net sterility increases with the advancing concentration, differed from concentration to concentration significantly ($P<0.05$).

Like the reduction in fertility and sterility, the per cent control over the reproduction also varies i.e. from 34.2 to 92.4 per cent and increasing with the advancing concentrations of bactospeine, differed signifcantly, from concentration to concentration ($P<0.05$) (Table – 24 ; Fig. -20).

4.3. C.b. Effect of bactospeine on reproduction under topical method:

Different concentrations of the bactospeine applied to the female under topical method delayed the sexual maturity significantly ($P<0.05$). In response to the females treatment under topical method with different concentrations of this bacterial preparation, the pre-oviposition period, varying from 2.64 to 3.62 days, tended to prolong with the advancing concentrations. But, the statistical analysis revealed that the concentrations from 0.05 to 0.10 per cent caused significantly less prolongation in pre-oviposition period as compared to the one per cent concentration ($P<0.05$) (Table – 22).

Similarly, under the topical method every concentration of the bactospeine also affected the oviposition period significantly ($P<0.05$). This period, varying from 2.65 to 5.46 days among different concentrations of this bacterial preparation applied topically and decreasing with the increasing concentration, was detected to differ with the concentration of the bactospeine applied to adults ($P<0.05$) (Table -22).

Different concentrations of the bactospeine applied to the female reduced considerably her fecundity ($P<0.05$). In response to the female's

treatment under topical method with different concentrations of this microbial preparation, the fecundity varied, from 77.8 to 132.6 eggs/ female and tending to decrease with the advancing concentration, depended significantly on the concentration of bactospeine. Further, every concentration of the bactospeine applied as residue film lead to reduction in hatching of eggs. In response to treatment of adults with different concentrations of bactospeine as residue films, the hatchability of eggs, varying from 23.3 to 62.7 per cent among different concentrations and tending indirectly proportional to the concentration was affected differently with the increasing concentrations of the residue film ($P<0.05$) (Table - 23).

Different concentrations of bactospeine applied topically to adults prolonged the incubation period as compared to the non-treatment condition ($P<0.05$). In response to adult's treatment under topical method with different concentrations of bactospeine, the incubation period varies from 3.16 to 4.82 days among employed concentrations. The incubation period (3.16 to 3.18 days) was affected identically by 0.05, 0.10 and 0.50 per cent ($P<0.05$) and it was also affected alike by 0.75 and 1.0 per cent concentrations, of course, with more prolongation ($P<0.05$) (Table-23).

Further, in response to adults treatment under topical method with different concentrations of the bactospeine the reduction in fecundity, net sterility and control over the reproduction varying from 26.8 to 66.2 per cent, from 12.76 to 59.34 per cent and from 34.3 to 90.5 per cent respectively and increasing with

Table - 24

Per cent reduction in fecundity, per cent net sterility and per cent control over reproduction in *D. obliqua* caused by "Bactospeine" under different modes of treatment.
(Values are mean ± S.E.)

Mode of treatment	Concentrations (%)	(%) Reduction in fecundity	(%)Net sterility	(%) Control over reproduction
L.D.M.	0.05	27.5	11.46	34.2
	0.10	31.2	15.76	44.2
	0.50	45.0	26.44	63.3
	0.75	59.4	32.73	79.8
	1.00	68.4	56.54	92.4
T.M.	0.05	26.8	12.76	34.3
	0.10	30.5	18.75	42.8
	0.50	42.1	20.63	60.4
	0.75	58.7	42.76	77.7
	1.00	66.2	59.34	90.5

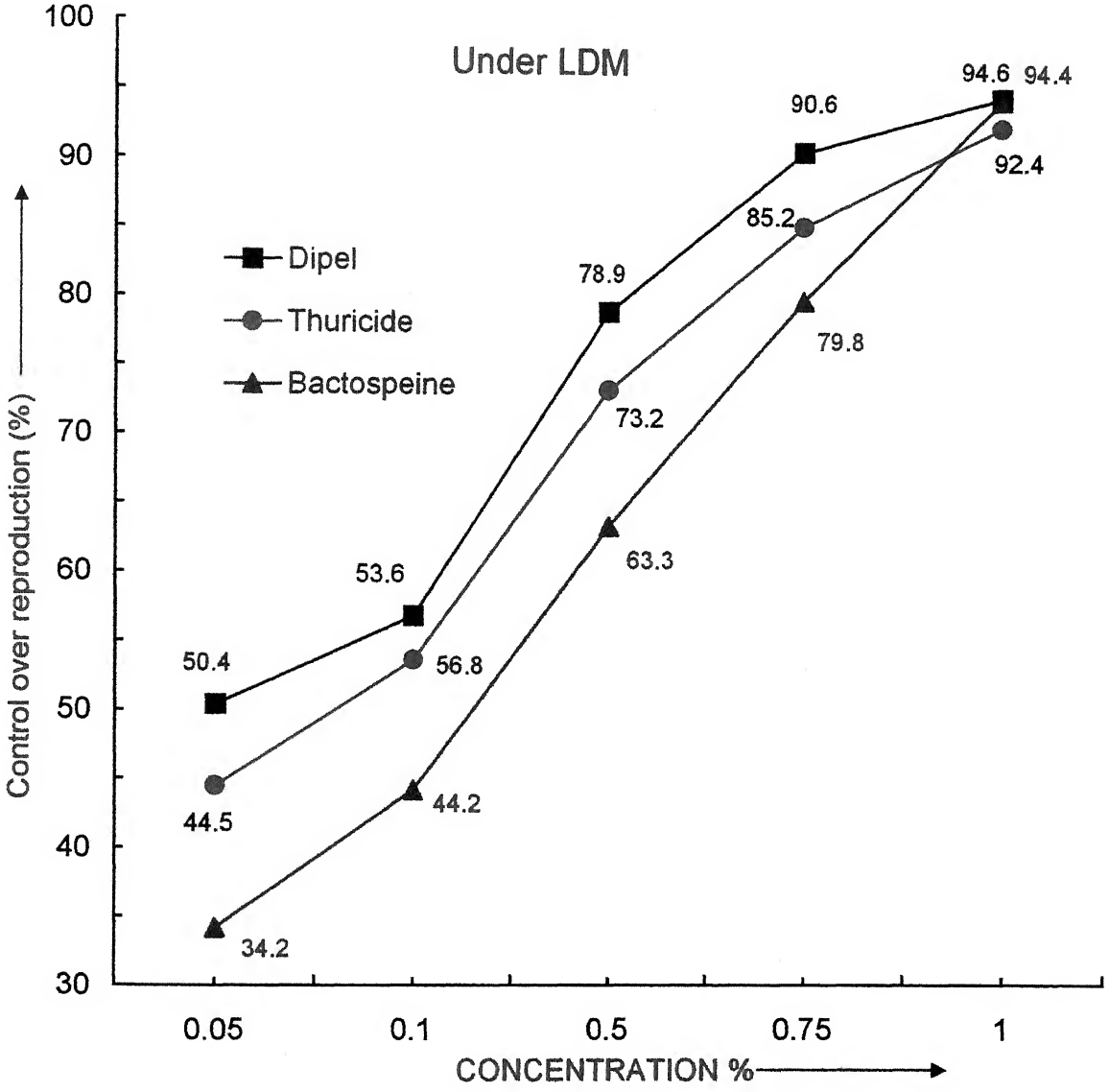


Figure 20. Per cent control over reproduction in *D. obliqua* caused by different concentrations of biological preparations under leaf dip method of treatment.

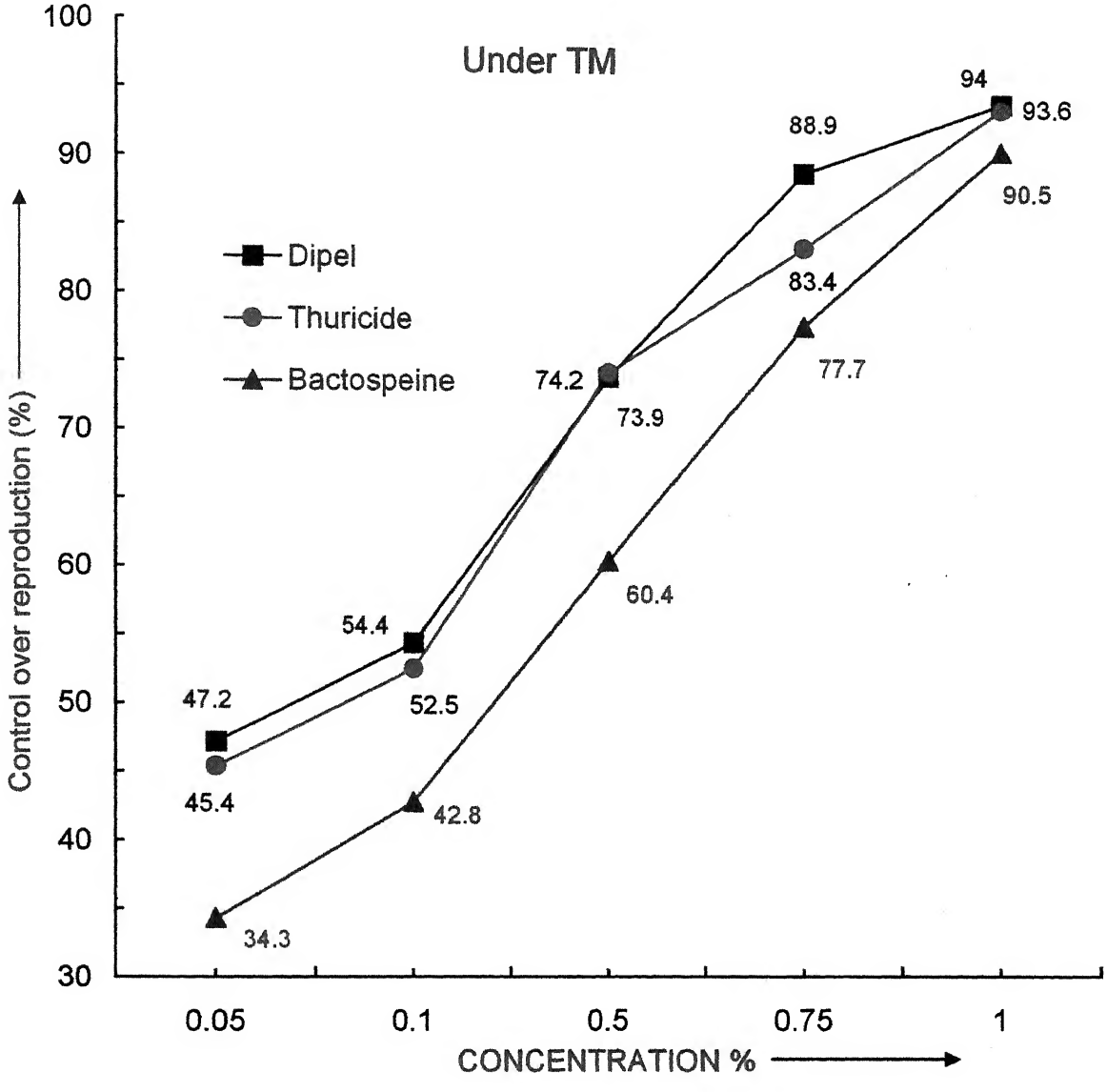


Figure 21. Per cent control over reproduction in *D. obliqua* caused by different concentrations of biological preparations under topical method of treatment.

the increasing concentration, differed significantly with concentration of the residue film of this microbial preparation ($P<0.05$) (Table -24 ; Fig. - 21).

The microbial preparations are highly toxic compounds which induced sterility and consequently, help in control of the pest population by reducing the fecundity and fertility. In this investigation, every microbial preparation, screened against *D. obliqua* found effective in causing sterility even at their lowest concentration. These microbial agents induce sterility by reducing the fecundity and by decreasing the viability of the eggs laid by a female. The related results reveal that the fecundity decreases with the increase in concentrations. The results also show that under the influence of the bacterial preparations, the fertility decreases distinctly with the advancing concentrations and the data pertaining to the per cent reduction in fecundity and per cent net sterility confirm the above facts. From the data, it is proved that dipel under leaf dip treatment cause maximum decline in the fecundity. However, the related data on the per cent reduction in the fecundity shows clearly that there is indirect proportionality between the reduction in the fecundity and concentrations of the thuricide and bactospeine. This trend is also evinced by the data relating to the per cent net fecundity caused by different concentrations of thuricide and bactospeine under the topical method of treatment. Under topical treatment also, the reduction in the number of the eggs laid by a female and the hatchabiltiy of the laid eggs are distinctly concentration dependent, these decrease with the increasing concentrations of the bacterial preparations. This trend is clearly

witnessed by the data on the per cent reduction in fecundity and per cent net sterility. Under the leaf dip method at 1.00 per cent concentration thd dipel induce about 86.38 per cent net sterility whereas at the same concentration, the thuricide and bactospeine induce about 72.35 and 56.54 per cent net sterility respectively, controlling the reproduction to the extent of 92.4 to 94.6 per cent.

The reports are also available in the literature that the bacterial preparations help in control of the lepidopterous pest population by reducing the fecundity and fertility. Jaques and Fox (1960); Heimpel (1961); Kulshrestha *et. al.* (1965); Dulmage and Martinez (1973); Govindrajan *et. al.* (1975); West *et. al.* (1997); Chandra *et.al.* (1999); Siegfried *et.al.* (2000); Chaturvedi (2002); Nault *et.al.* (2000) ; Bajpai (2003) Li *et al.* (2005), Vastrad (2005) and Kumar and Gujar (2005) also worked on the effect of the microbial preparations on the development of different insects. As per results of this investigation, *D. obliqua* a lepidopterous pest, respond fairly well at 1.0 per cent concentration to the bacterial preparations applied by leaf dip method or topically. With this concentration level every bacterial preparation used in this investigation induces more than 50 per cent sterility. And these findings support the works of above workers. However, El-Guindy and Bishara (1975) and Cantwell *et al.*(1986) did not favour the findings of this investigation.

The bacterial preparations applied through leaf dip method and topical method affect the pre-oviposition and oviposition period. The pre-oviposition period prolongs even with 0.05 per cent concentration of any used

microbial preparation. This fact suggests that a microbial preparation delays the sexual maturity, increases with the increasing concentration of bacterial preparation. In context of the delay in sexual maturity, considering influence of 1.0 per cent concentration, the different microbial preparations may be arranged as dipel, thuricide and bactospeine in descending order.

Like the pre-oviposition period, each microbial preparation used in this investigation even at its lowest concentration (0.05 per cent) affects the oviposition period too and, this period decreases with the increasing concentrations of used biopesticides. Since the decreased oviposition period is associated with the decreased fecundity and fertility, this fact supports that every bacterial preparation retards or inhibits the oviposition.

Results show that each microbial preparation has potential to increase the incubation period. Every concentration of dipel, thuricide and bactospeine exert influence on the incubation period of *D. obliqua*. However, 0.75 and 1.0 per cent concentration of different microbial preparations exert more influence on incubation period. The different concentrations of microbial perparations cause proportionate increase in the incubation period depending on their strength. Dipel (1.0% concentration) under leaf dip treatment extends the incubation period upto 5.42 days while under natural conditions it was noted only 2.78 days. The above facts suggest that the bacterial preparations lower the speed of the embryonic development which becomes more and more slow with the progressive increase in the strength of the bacterial preparation.

The literature reveals that among lepidopterous pests, the treatment by leaf dip method with different bacterial insecticides has led to the adverse effect on reproduction i.e., it leads to the sterility with different levels of success, Howland *et.al* (1965); Toppozada *et.al.* (1966); Henneberry and Kishaba (1966); Sotto and Graves (1967); El-Guindy and Bishara (1975); Bobaye and Carman (1975); Flint and Smith (1977); Calkins *et.al.* (1977); Flint *et.al.*, (1978); Wright *et.al.* (1980); Segistan *et.al.* (1982); Abdel *et.al.* (1985); Morris (1988); Sharma (1993); Navon *et.al.* (1994); Trisyono and Whalon (1997); Siegfried *et.al* (2000); Navrozidis *et.al.* (2000); Nault *et.al.*(2000); Chaturvedi (2002) ; Bajpai (2003); Fetoh and Azazy (2004) ; Devaki and Krishnayya (2004) ; Li *et al.* (2005) ; Hernadez *et al.* (2005) and Vastrad (2005) evaluated the effect of the insecticides and biological preparations on different insects and reported that these controlling agents induce the sterility in adults. Our results also indicate that when bacterial preparations are administerd in adults, they are able to induce the sterility which exhibits direct proportionality to their concentration. At 1.0 per cent concentration all the three bacterial preparations used in this investigation are able to cause very high sterility but not cent per cent. At this concentration level their sterility inducing potential differs among them and on this basis, these can be arranged as dipel (86.38%), thuricide (72.35%) and bactospeine (56.54%) in descending order. However, in case of all the bacterial preparations, there is a progressive increase in the sterility with the advancing concentrations which decrease the fecundity and fertility accordingly. As per our

results, at 1.00 per cent concentration of a microbial preparation which induces very high sterility, the longevity of the adults is also very much reduced.

All the three bacterial preparaions screened under this investigation are able to control the reproduction in *D. obliqua* to the extent of 90.5 to 94.6 per cent at 1.0 per cent concentration and in this respect, at 1.0 per cent concentration the dipel is the most effective bacterial preparation and bactospeine which exert similar influence in controlling the reproduction is the least effective one under topical method of treatment. However, the dipel is most effective under leaf dip method of treatment. Thuricide is also able to control the reproduction in *D. obliqua* to the extent of about 94.4 per cent.

The comparative sterilizing influene of a bacterial preparation under both methods of treatment is quite distinct at 1.0 per cent concentration. The results pertaining to the per cent sterility induced by a microbial preparation, suggest that dipel, thuricide and bactospeine are more effective under leaf dip administration in adults than its application topically to adults.

4.4. STERILITY EFFECT OF BACTERIAL PREPARATIONS ON SEXES:

4.4A. Sterility effect of dipel on male and female *D. obliqua* :

The mating between the untreated female and treated male moth, resulted in far reduced fecundity (92.5 eggs/female) as compared the mating between untreated male and untreated female (346.2 eggs/female) and it caused

54.4 per cent sterility. The hatching of eggs also affected significantly ($P<0.05$). The mating between treated female and untreated male moth caused a reduction in fecundity and hatchability (81.7 eggs/female; 38.52 per cent hatching of eggs). These results are also very significant in comparison to earlier experiment and control experiment ($P<0.05$). The mating between treated female and treated male inducing fecundity (70.4 eggs/female) and this finding is significantly different from above experiments ($P<0.05$). Eggs obtained from this pair show very poor percentage of hatching (15.71%). The net sterility also increased (72.7%) in comparison of above experiments (Table- 25).

4.4. B. Sterility effect of thuricide on male and female *D. obliqua:*

The cross between untreated female and treated male caused fall in fecundity (91.4 eggs/female) as compared to control experiment i.e. between untreated female and untreated male (346.2 eggs/female). The eggs obtained from this pair cause 46.15 per cent hatching. The mating between treated female and untreated male reduced the fecundity (84.6 eggs/female). The fertility was also reduced (38.55%) significantly. The mating between the treated male and treated female caused fall in the fecundity (78.4 eggs/female) and fertility (20.51) per cent and the per cent net sterility also increased (65.43 per cent) in comparison of other experiments (Table- 26).

4.4.C. Sterility effect of bactospeine on male and female *D. obliqua :*

Table - 25

Sex specific effect of "Dipel" on reproduction in *D. obliqua*.
(Values are mean ± S.E.)

Mating between	No. of eggs laid (mean ±S.E.)	No. of eggs hatched (mean ±S.E.)	(%) Hatching	(%) Net sterility
UNT F · TR M	92.5±4.34	42.4±2.14	45.65	54.5
TR F · UNT M	81.7 ±2.35	31.4±2.63	38.52	61.3
TR FXTR M	70.4±4.36	11.2±1.35	15.71	72.7
UNT F · UNT M (control)	346.2±4.24	310.3±2.14	89.60	–

Table - 26

Sex specific effect of "Thuricide" on reproduction in *D. obliqua*.
(Values are mean ± S.E.)

Mating between	No. of eggs laid (mean ±S.E.	No. of eggs hatched (mean ±S.E.)	(%) Hatching	(%) Net sterility
UNT F · TR M	91.4±4.22	42.3±2.13	46.15	50.62
TR F · UNT M	84.6±3.76	32.6±3.13	38.55	56.72
TR FXTR M	78.4±4.25	16.6±2.14	20.51	65.43
UNT F · UNT M (control)	346.2±4.24	310.3±2.14	89.60	–

Table - 27

Sex specific effect of "Bactospeine" on reproduction in *D. obliqua*.
(Values are mean ± S.E.)

Mating between	No. of eggs laid (mean ±S.E.)	No. of eggs hatched (mean ±S.E.)	(%) Hatching	(%) Net sterility
UNT F · TR M	109.2±4.42	61.42±1.22	55.96	46.32
TR F · UNT M	89.2±3.28	42.42±0.46	46.67	52.32
TR FXTR M	84.0±4.36	21.42±0.14	25.12	60.24
UNT F · UNT M (control)	346.2±4.24	310.3±2.14	89.60	–

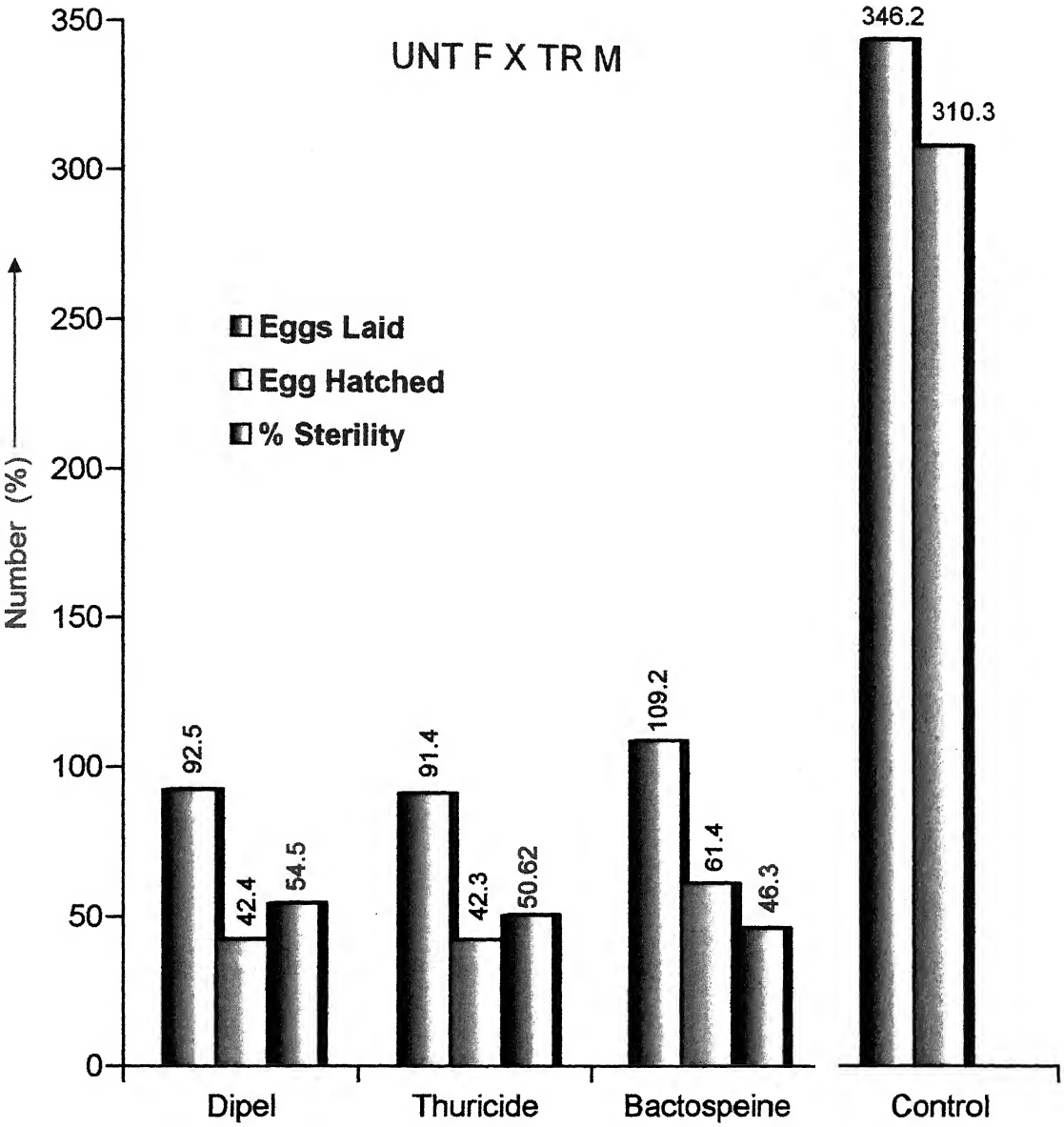


Figure 22. Effect of biological preparations on reproduction of *D. obliqua* (between UNT F x TRM)

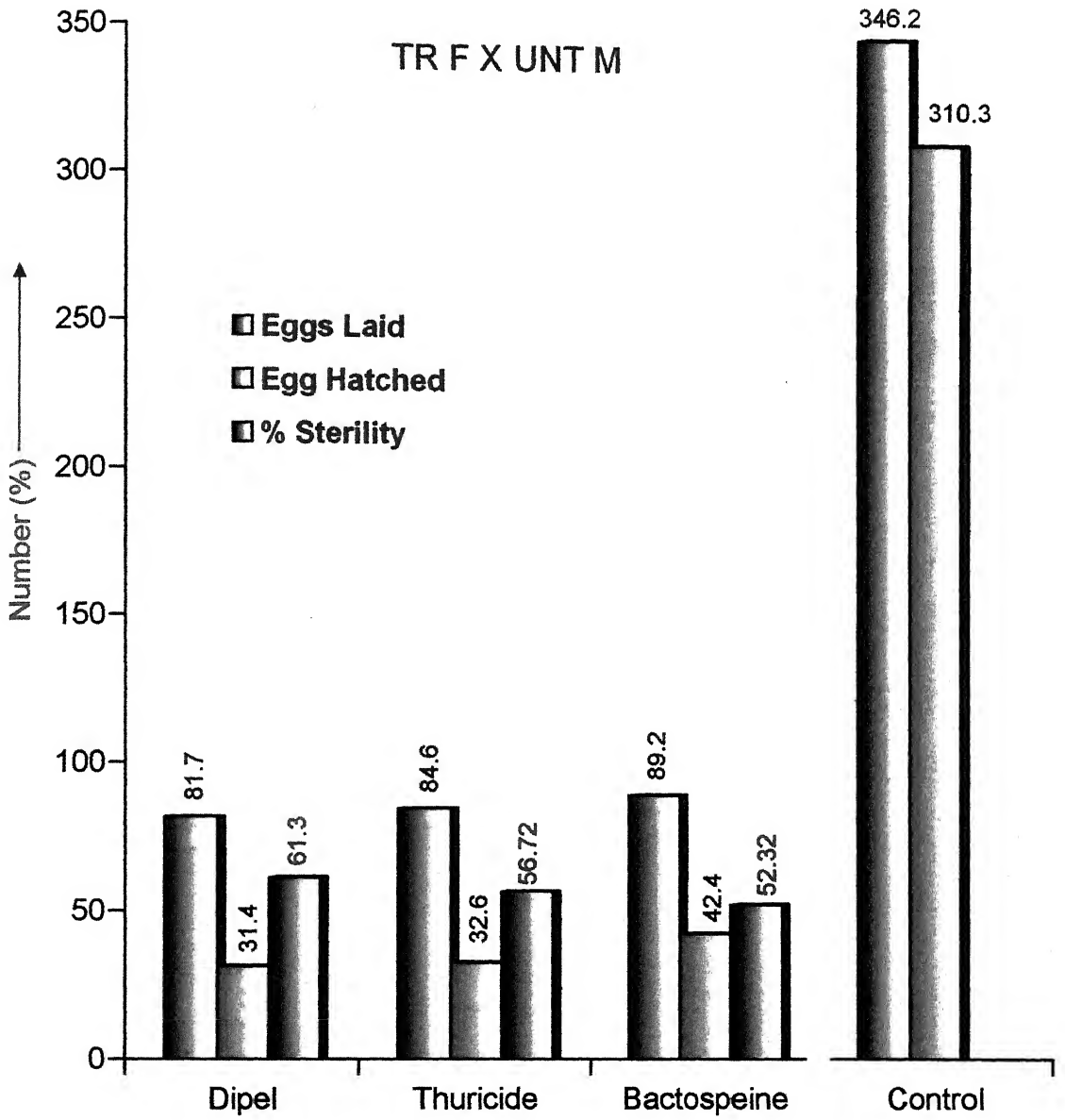


Figure 23. Effect of biological preparations on reproduction of *D. obliqua* (between TRF x UNTM)

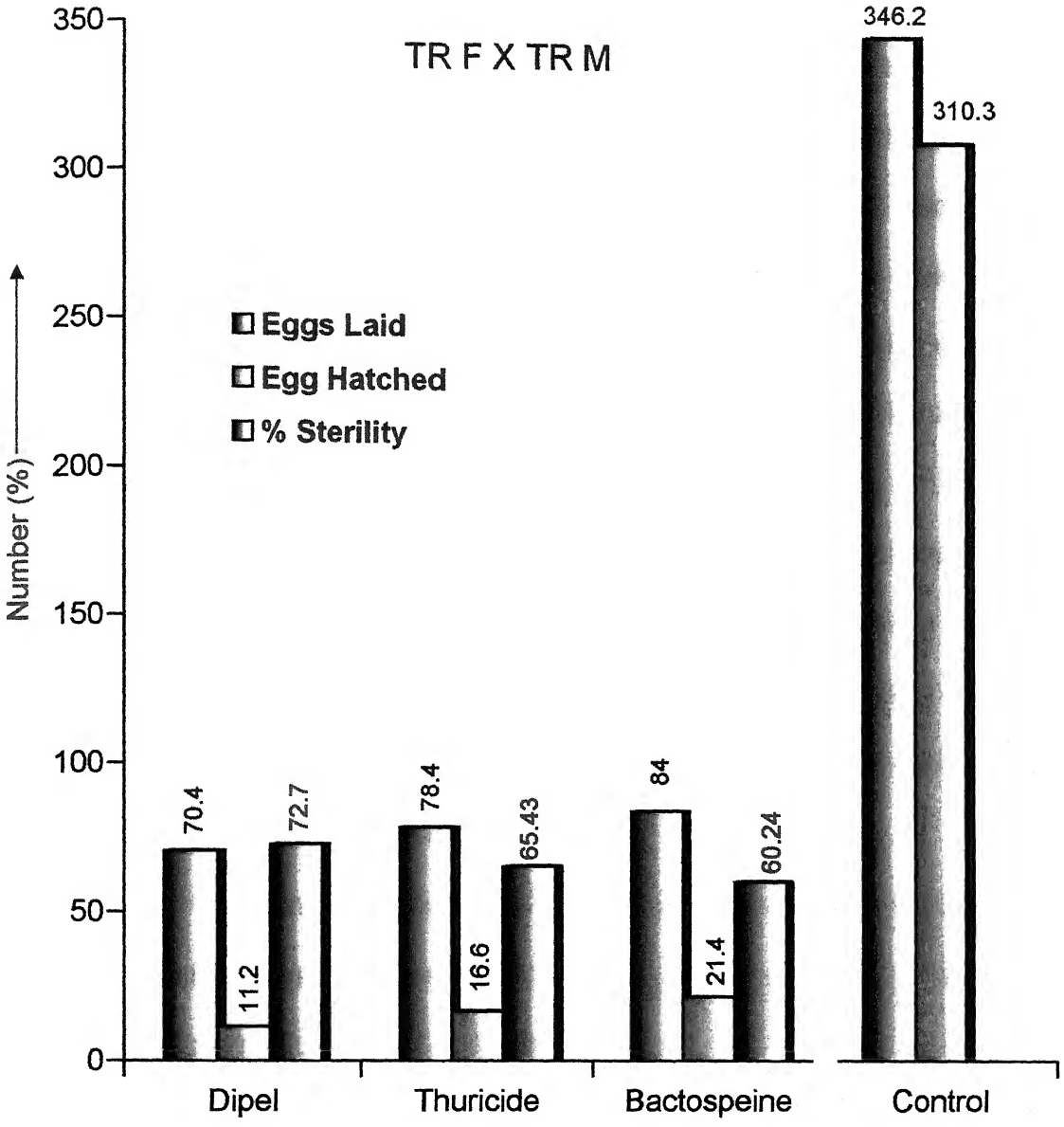


Figure 24. Effect of biological preparations on reproduction of *D. obliqua* (between TRF x TRM)

The mating between untreated female and treated male caused reduction in the fecundity (109.2 eggs/female) as compared to the mating between untreated male and untreated female (346.2 eggs/female). But mating between treated female and untreated male caused more reduction in the fecundity (89.2 eggs/female). While mating between treated male and treated female caused a more decline in the fecundity (84.0 eggs/female). Like the fecundity, the fertility also differed significantly. The eggs obtained after the mating between untreated female and treated male, hatched only 55.96 per cent. The hatching percentage decline further (46.67%) in eggs obtained after mating between treated female and untreated male. Futher, the hatching recorded only 25.12 per cent in that eggs, were obtained after mating between treated male and treated female. The per cent net sterility also increased (60.24%) in comparison to other experiments (Table – 27).

Among three bacterial preparations used in this investigation dipel shows best results regarding sterility. In all cases microbial preparations exert more sterilizing influence in the female *D. obliqua* (Fig. – 22, 23, 24).

In different pests, the sex oriented sterilizing influence of insecticides has been reported by a good number of workers (Crystal (1965); Kaloostain (1970); Chaturvedi (2002); Bajpai (2003) Bakr (2004) and Li *et al.* (2005). This investigation also reveals the sex specific sterilizing influence of these bacterial preparations in *D. obliqua* too. The results pertaining to the sterility of *D. obliqua* obtained from the crosses between the treated male and the

untreated female, between the untreated male and the treated female and between the treated male and the treated female show four facts: (a) Every bacterial preparation used in this investigation induce the sterility in both sexes. (b) The cross between the treated male and treated female induces more sterility than that of a cross in which only one sex is treated. (c) In inducing sterility, the bacterial preparations are differently effective in male and female. (d) The cross between the treated female and untreated male cause more sterility than that of a cross between untreated female and treated male.

Similar findings have also been reported earlier by Sharma (1993) by using insect growth regulators to observe the effect of these on development and sterility of *U. pulchella*. Bobaye and Carman (1975); Chaturvedi (2002); Bajpai (2003); Hernadez *et al.* (2005) ; Vastrad *et al.* (2005) and Kumar and Gujar (2005) have also reported similar findings. Hence, the present findings were also found to corroborate with the results of the earlier researchers.

4.5. Compatibility of Dipel with insedticides against the larvae (five days old) of *D. obliqua:*

Experimental findings of bioassay presented in tables from 28 to 42 and divulge the toxicity of Endosulfan, BHC, Malathion, Quinalphos, Cypermathrin and Fenvelerate, respectively against the five days old larvae of *D. obliqua.* As per data presented in table –34 the relative toxicity and toxicity index of all the six insecticides tested, shows that Cypermathrin was the most toxic insecticide followed by Fenvelerate, Quinalphos, Endosulfan, Malathion and

Table – 28

Toxicity of different concentrations of Endosulfan against five days old larvae of *D. obliqua*.

Sl. No.	Concentration used (%)	No. of larvae used per replicate	Av. No. of larvae killed	Corrected % kill	LC_{50}	Fiducial limit
1.	0.05	20	4.1±0.32	20.68		
2.	0.04	20	17.2±0.14	86.20		0.0185 (u)*
3.	0.03	20	16.5±0.26	82.75	0.01380	
4.	0.02	20	11.7±0.15	58.62		0.01025 (L)**
5.	0.01	20	5.5±0.14	27.58		

* U = upper limit

**L = lower limit

Table – 29

Toxicity of different concentrations of BHC against five days old larvae of *D. obliqua*.

Sl. No.	Concentration used (%)	No. of larvae used per replicate	Av. No. of larvae killed	Corrected (%) kill	LC_{50}	Fiducial limit
1.	0.025	20	4.6±0.14	23.33		
2.	0.20	20	17.3±0.15	86.66		0.09367 (U)*
3.	0.15	20	14.6±0.14	73.33	0.07310	
4.	0.10	20	12.0±0.13	60.00		0.065552 (L)**
5.	0.05	20	5.3±0.16	26.60		

* U= Upper limit

**L = Lower limit

Table – 30

Toxicity of different concentrations of Malathion against five days old larvae of *D. obliqua*.

Sl. No.	Concentration used (%)	No. of larvae used per replicate	Av. No. of larvae killed	Corrected (%) kill	LC_{50}	Fiducial limit
1.	0.005	20	4.0±0.12	20.00		0.01175 (L)**
2.	0.04	20	16.0±0.14	80.00		
3.	0.03	20	14.6±0.14	73.33	0.01652	0.02310 (U)*
4.	0.02	20	10.0±0.15	50.00		
5.	0.01	20	5.3±0.16	26.66		

* U= Upper limit

**L = Lower limit

Table – 31

Toxicity of different concentrations of Quinalphos against five days old larvae of *D. obliqua*.

Sl. No.	Concentration used (%)	No. of larvae used per replicate	Av. No. of larvae killed	Corrected (%) kill	LC_{50}	Fiducial limit
1.	0.0025	20	4.0±0.12	20		0.005811 (L)**
2.	0.02	20	16.0±0.12	80		0.00958 (U)*
3.	0.015	20	14.0±0.14	70	0.01754	
4.	0.01	20	12.0±0.16	60		
5.	0.005	20	6.0±0.15	30		

*U = Upper limit

**L= Lower limit

Table – 32

Toxicity of different concentrations of Cypermathrin against five days old larvae of *D. obliqua*.

Sl. No.	Concentration used (%)	No. of larvae used per replicate	Av. No. of larvae killed	Corrected (%) kill	LC_{50}	Fiducial Limit
1.	0.004	20	17.3±0.14	86.66		0.001751 (U)*
2.	0.003	20	16.0±0.15	80.00		
3.	0.002	20	12.6±0.12	63.33	0.00152	
4.	0.001	20	6.0±0.0.15	30.00		0.001151 (L)**
5.	0.0005	20	3.3±0.04	16.66		

* =U Upper limit

**L = Lower limit

Table – 33

Toxicity of different concentrations of Fenvelerate against five days old larvae of *D. obliqua*.

Sl. No.	Concentration used	No. of larvae used per replicate	Av. No. of larvae killed	Corrected (%) kill	LC_{50}	Fiducial limit
1.	0.004	20	16.7±0.12	83.66		0.00208 (U)*
2.	0.003	20	13.3±0.14	66.66		
3.	0.002	20	10.6±0.16	53.33	0.00162	
4.	0.001	20	6.6±0.02	33.33		
5.	0.0005	20	4.0±0.05	20.00		0.001206 (L)**

*U = Upper limit

**L = Lower limit

Table – 34

Relative toxicity of different insecticides against the larvae (five days old) of *D. obliqua*.

Sl. No.	Name of insecticides	Regression equation	X^2	LC_{50}	Toxicity index	Log 10^n
1.	Endosulfan	Y=1.874 x +(-0.881)	4.727	0.01380	1.20	10^5
2.	BHC	Y=1.929 x +0.5126	5.152	0.07310	0.23	10^4
3.	Malathion	Y=1.476 x +1.728	7.65	0.01652	1.00	10^4
4.	Quinalphos	Y=2.093x +1.079	1.697	0.01754	2.21	10^4
5.	Fenvelerate	Y=1.829 x +0.974	0.560	0.00152	10.36	10^5
6.	Cypermathrin	Y=2.42x + (-0.208)	1.08	0.00162	11.60	10^5

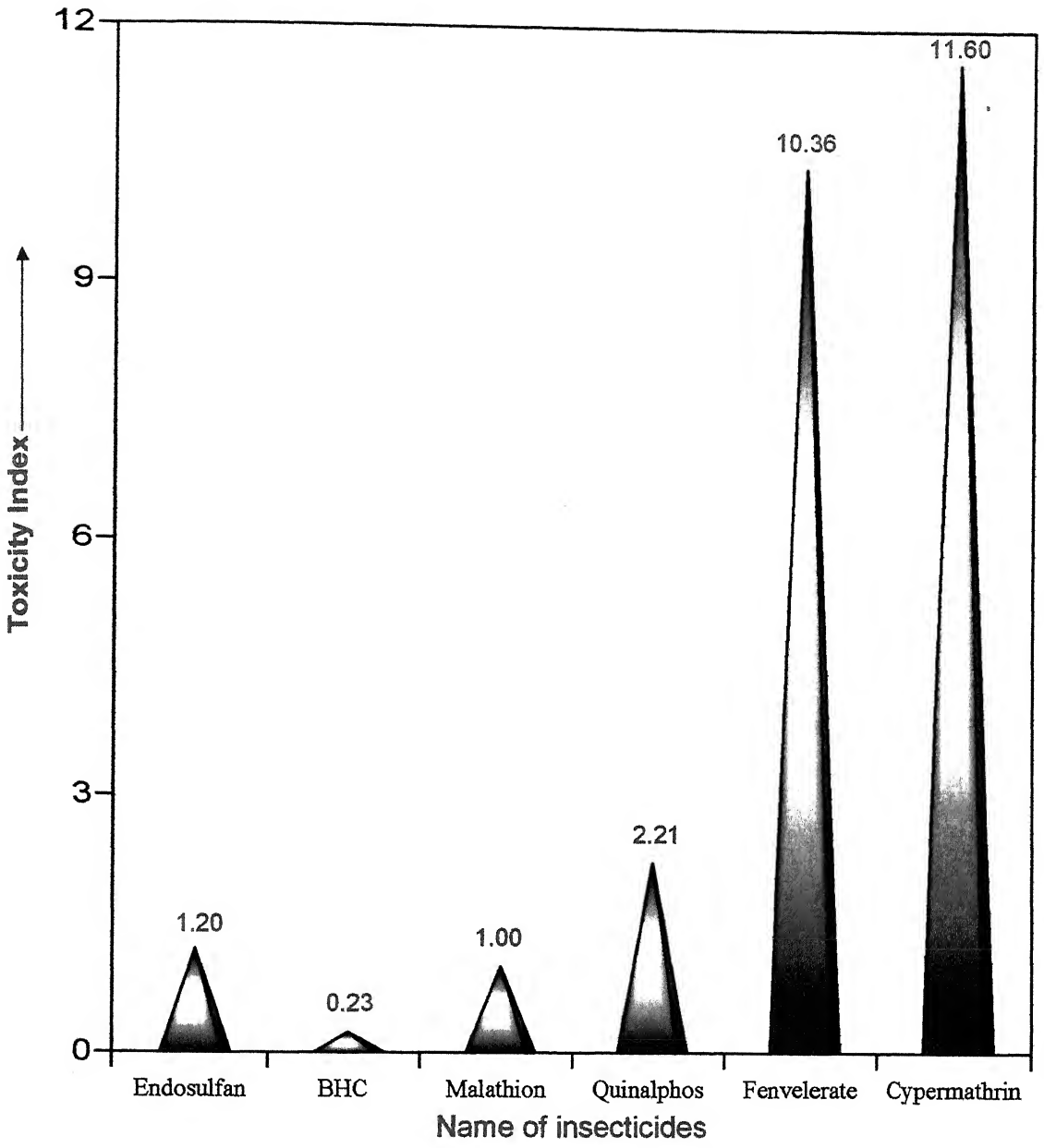


Figure 25. Relative toxicity of different insecticides against the larvae (five days old) of *D. obliqua.*

Table – 35

Toxicity of different concentrations of Endosulfan + Dipel against five days old larvae of *D. obliqua*.

Sl. No.	Concentration used (%)	No. of Larvae used per replicate	Av. No. of Larvae died	Corrected per cent kill	LC_{50}	Fiducial limit
1.	0.04	20	17.3 ±0.12	86.66		0.01312 (U)*
2.	0.03	20	15.3±0.16	76.66		
3.	0.02	20	13.3±0.12	66.66	0.00635	
4.	0.01	20	10.6±0.14	53.33		0.00306 (L)**
5.	0.005	20	10.0±0.14	50.00		

* U= Upper limit

**L = Lower limit

Table – 36

Toxicity of different concentrations of BHC + Dipel against five days old larvae of *D. obliqua*.

Sl. No.	Concentration used (%)	No. of Larvae used per replicate	Av.No. of Larvae died	Corrected per cent kill	LC_{50}	Fiducial Limit
1.	0.20	20	17.9 ± 0.16	89.65		0.0556 (U)*
2.	0.15	20	16.5 ± 0.12	82.75		
3.	0.10	20	13.7 ± 0.15	68.96	0.03116	0.031181 (L)**
4.	0.05	20	13.1 ± 0.12	65.51		
5.	0.025	20	8.9 ± 0.14	44.82		

*U = Upper limit

**L= Lower limit

Table – 37

Toxicity of different concentrations of Malathion + Dipel against five days old larvae of *D. obliqua*.

Sl. No.	Concentration used (%)	No. of Larvae used per replicate	Av.No. of Larvae died	Corrected per cent kill	LC_{50}	Fiducial Limit
1.	0.04	20	18.6±0.14	93.33		0.01128 (U)*
2.	0.03	20	16.6±0.15	83.33		
3.	0.02	20	13.3 ±0.13	66.66	0.00742	
4.	0.01	20	12.0±0.13	60.00		
5.	0.005	20	9.3±0.12	46.66		0.0048 (L)**

* U= Upper limit

**L = Lower limit

Table – 38

Toxicity of different concentrations of Quinalphos + Dipel against five days old larvae of *D. obliqua*.

Sl. No.	Concentration used	No. of Larvae used per replicate	Av. No. of Larvae died	Corrected per cent kill	LC_{50}	Fiducial Limit
1.	0.02	20	18.0±0.12	90.00		0.004378 (U)*
2.	0.15	20	17.3±0.14	86.66		
3.	0.01	20	16.6±0.16	83.30	0.002218	
4.	0.005	20	13.3±0.14	66.66		0.001242 (L)**
5.	0.0025	20	10.6±0.12	53.33		

*U = Upper limit

**L = Lower limit

Table – 39

Toxicity of different concentrations of Fenvelerate + Dipel against five days old larvae of *D. obliqua*.

Sl. No.	Concentration used (%)	No. of Larvae used per replicate	Av. No. of Larvae died	Corrected per cent kill	LC_{50}	Fiducial limit
1.	0.004	20	17.9±0.16	89.65		0.00108 (U)*
2.	0.003	20	17.2±0.15	86.20		
3.	0.002	20	16.5±0.14	82.75	0.00074	
4.	0.001	20	11.7±0.12	58.62		
5.	0.0005	20	8.9±0.13	44.82		0.00048 (L)**

*U = Upper limit

**L= Lower limit

Table – 40

Toxicity of different concentrations of Cypermathrin + Dipel against five days old larvae of *D. obliqua*.

Sl. No.	Concentration used (%)	No. of Larvae used per replicate	Av. No. of Larvae died	Corrected per cent kill	LC_{50}	Fiducial limit
1.	0.004	20	18.0±0.15	90.00		0.00108 (U)*
2.	0.003	20	16.6±0.14	83.33		
3.	0.002	20	16.0±0.13	80.00	0.00069	
4.	0.001	20	10.0±0.15	50.00		
5.	0.0005	20	9.3±0.12	46.66		0.000443 (L)**

*U = Upper limit

**L = Lower limit

Table – 41

Relative toxicity of different insecticides in combination with Dipel against five days old larvae of *D. obliqua*.

Sl. No.	Name of insecticides	Regression equation	X^2	LC_{50}	Toxicity index	Log 10^n
1.	Endosulfan +Dipel	=1.068 x +2.007	2.12	0.00635	2.599	10^{-5}
2.	BHC +Dipel	=1.356x+1.617	1.291	0.031161	0.528	10^{-4}
3.	Malathion + Dipel	1.742x+1.74	0.489	0.00742	2.227	10^{-4}
4.	Quinalphos + Dipel	=1.294x+3.258	0.190	0.002218	7.45	10^{-4}
5.	Fenvelerate + Dipel	=1.83x+1.590	0.865	0.00074	22.57	10^{-5}
6.	Cypermathrin + Dipel	=1.498x +2.270	0.501	0.000690	24.199	10^{-5}

Note: Toxicity index has been calculated by taking LC_{50} value of Malathion as unit.

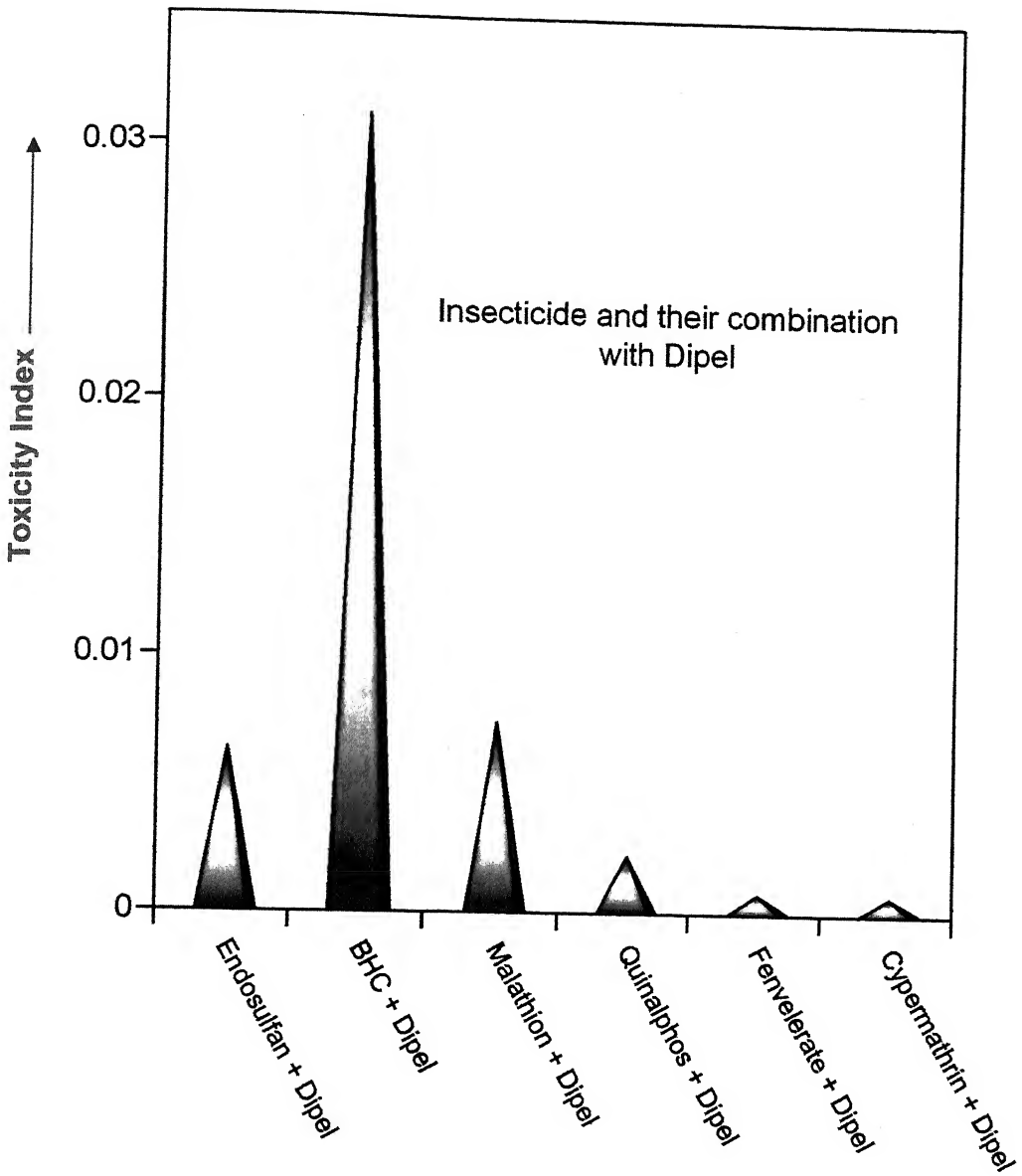


Figure 26. Effect of Dipel on the toxicity of insecticides against the larvae of *D. obliqua* showing Lc_{50} values.

Table – 42

Effect of Dipel on the toxicity of insecticides against the larvae of *D. obliqua*.

S.No.	Insecticides and their combination with *B. thuringiensis*	Lc_{50}	Toxicity index
1.	Endosulfan	0.01380	-
2.	Endosulfan + Dipel	0.00635	2.160
3.	B H C	0.07310	-
4.	B H C + Dipel	0.03116	2.314
5.	Malathion	0.01652	-
6.	Malathion + Dipel	0.00742	2.228
7.	Quinalphos	0.01754	-
8.	Quinalphos + Dipel	0.002218	3.375
9.	Fenvelerate	0.00162	-
10.	Fenvelerate + Dipel	0.00074	2.179
11.	Cypermathrin	0.00152	-
12.	Cypermathrin + Dipel	0.000690	2.086

BHC. It was also found that there is a negative co-relation between the LC_{50} values and toxicity index of the insecticides evaluated. The degree of toxicity of Cypermathrin (11.60), Fenvelerate (10.36), Quinalphos (2.21), Endosulfan (1.20), Malathion (1.00) and BHC (0.23) times respectively was calculated on the basis of bioefficacy test, the toxicity of insecticides can be arranged in the following descending order (Fig. – 25).

Cypermathrin>Fenvelerate> Quinalphos> Endosulfan> Malathion> BHC.

As regard the effectivity of insecticides in combination with sub-lethal concentration of Dipel, the data presented in tables from 35 to 40 showed increased toxicity of each insecticide. Results showed that combination of bacterial toxin with lower concentrations of each insecticide result in high rate of larval mortality, which ultimately reflects the lower LC_{50} values of these insecticides.

Considering the relative toxicity of Cypermathrin, Fenvelerate, Quinalphos, Endosulfan, Malathion and BHC in combination with Dipel it was calculated on 24.199, 22.57, 7.45, 2.599, 2.227 and 0.528 times, respectively, and it was found more toxic than malathion. However, their sequence of toxicity remained unchanged (Table – 41).

As far as the effect of dipel on the toxicity of insecticides is concerned, the data, cited in table - 42 showed the marked influence of the toxicity of insecticides. It contributed (3.37 times) toxicity when mixed with quinalphos, whereas it was minimum (2.085 times) in combination with

cypermathrin. The response of *B. thuringiensis* with insecticides, showed the following order of toxicity.

Quinalphos>BHC>Malathion, Endosulfan>Fenvelerate> Cypermathrin.

From the above mentioned data (results) it could be concluded the mixing of sub-lethal concentrations of dipel with insecticides increases their toxicity considerably but interestingly its effect was more pronounced with insecticide having low order of toxicity except quinalphos (Table –42 ; Fig. - 26).

Results of the screening of six insecticides against the five days old larvae of *D. obliqua* make it apparent that insecticides which belong to synthetic pyrethroid group were more toxic than any other group of insecticides. The most toxic compound recorded was cypermathrin and it was closely followed by Fenvelerate, Malathion and BHC proved to be the least toxic to the five days old larvae of *D. obliqua*.

Pandey *et.al.* (1980) studied relative toxicity of different insecticides belonging to organochlorine and organophosphate groups against fourth instar larvae of *P. ricini* and found quinalphos comparatively more toxic than malathion and endosulfan. Thus findings of this study corroborate with the reports of earlier workers.

Chen *et.al*, (1983); and Tiwari (1985) reported higher toxicity of cypermathrin. In the present study too, the higher toxicity of cypermathrin registered a further link in this direction. As regards the toxicity of synthetic pyrethroids against *D. obliqua*, nothing appears in the available literature.

However, several reports on the efficacy of these insecticides against many lepidopterans are printed in different journals.

Likewise, no information is available in records on the combined use of insecticides and *B. thuringiensis* preparations against *D. obliqua.* However, there are many reports which indicate that amalgamation of bacterium increased the toxicity of insecticides to a greater extent (Creighton *et.al.*, 1972, Shekhar and Joshi, 1984; Chaturvedi; 2002 and Bajpai, 2003).

This study reveals that sub-lethal concentrations of dipel in combination with all six insecticides resulted in decrease of LC_{50} values to varying degree. This shows that combination of sub-lethal concentrations of the bacterium with an insecticide enhances the toxicity and, therefore, sub-lethal doses of insecticides mainly below median lethal doses could be utilized to give an effective kill than to that of median lethal dose when used alone.

As regards the toxicity of insecticides in combination with dipel, it is also obvious from the present findings that combination of dipel with synthetic pyrethroids has showed higher toxicity against the *D. obliqua*. Similar findings were also reported by Shekhar and Joshi (1984) and Joshi and Bharadwaj (1987) and Chaturvedi (2002) against *T. ni.* ; *S. litura* and *U. pulchella* respectively.

Considering the toxicity of organophsphates with *B. thuringiensis*, Shekhar and Joshi (1984) and Joshi and Bharadwaj (1987) reported that quinalphos and malathion in combination with bacterial preparation produced a

synergistic effect against *T. ni & S. litura,* respectively. Therefore, findings of this study corroborate with the findings of earlier workers, but contradict with the findings for Godavaribai *et. al.* (1962) and Benz (1971) who reported that malathion produces antagonistic effect with the pathogen.

As regards the toxicity of organochlorine insecticides with *B. thuringiensis*, Sogoyan and Solobodyanyuk (1980) recorded the response of *B. thuringiensis* against *G. mellonella and B. mori.* Thus, increased toxicity of BHC and endosulfan with dipel against the five days old larvae of *D. obliqua* are in accordance with the findings of earlier workers. The increased toxicity of endosulfan in combination with bacterium has also been observed by Joshi and Bharadwaj (1987); Bajpai (2003) ; Devaki and Krshnayya (2004) and Vastrad *et al.* (2005), which further strengthen the results of present study.

In addition to above, experimental findings also indicate that the combination of dipel with lower concentration of each insecticides results in higher larval mortality, whereas the same concentration of dipel with higher concentration of each insecticide did not prove as effective as the former combinations. Probably, higher concentration of insecticide reduces the efficacy of dipel when used in sub-lethal concentration against the larvae of *D. obliqua*. This finding gets support from the findings of Morris (1972) who reported that combination of thuricide with higher concentrations of malathion and pyrethrum caused an antagonistic effect against *H. cunea.*

As regards the effect of dipel on the toxicity of insecticides, the findings elicit that mixing of dipel with insecticides resulted in increased toxicity of each tested insecticide, though maximum response was imparted to quinalphos. This finding is in complete agreement with the findings of Joshi and Bharadwaj (1987); Chaturvedi (2003); Bajpai (2003) and Vastrad *et al.* (2005). They reported that combination of *B. thuringiensis* with quinalphos yielded highest toxicity index when compared with synthetic pyrethorids and other insecticides against the larvae of *S. litura* and *U.pulchella* respectively. It is also evident from the findings that response of dipel was more pronounced with those insecticides whose median lethal concentration was higher against the *D. obliqua*. The similar findings were reported by Joshi and Bharadwaj (1987); Chaturvedi (2003); Bajpai (2003) and Naglaa *et al.* (2004), which strengthen the findings of present investigation.

The present research work reveals that the bacterial preparations (Dipel, Thuricide, Bactospeine) have proved to be best controlling agents without disturbing our ecosystem. These biological preparations applied against *D. obliqua* by leaf dip method and topical method, causing remarkable mortality in larvae. They affect the growth and development of *D. obliqua*. Various concentrations of each bacterial preparations affect significantly in different experiments. The results show prolongation of larval and pupal period. Loss of weight in larvae and pupae also was observed. Weight loss might be due to irritation in the body by the action of bacterial preparation. The fecundity and

fertility also affected differently. So these results are significant in the pest management programme as damage by the pest will be reduced substaintially due to its manifold interaction. Various degree of morphological abnormalities were also marked. Development of normal adults were greatly affected due to use of dipel, thuricide, bactospeine. These factors keep the pest population below economic threshold. Compatibility of dipel with different insecticides against the larvae of *D. obliqua* also studied. The study clears that sub lethal concentration of dipel in combination with all six insecticides resulted in decrease of LC_{50} values. This finding shows that combination of sub-lethal concentration of *B. thuringiensis* with an insecticide enhances the toxicity. Therefore, low doses of such combinations could be utilized to give an effective control of *D. obliqua.*

It is obvious from the findings and foregoing discussion that amongst all the three formulations of *B. thuringiensis*, Dipel is the most effective treatment against the larvae of *D. obliqua* and when it is mixed with Cypermethrin, it offers the maximum kill of the test insect.

In view of the over all studies made, It can be concluded that larvae of *D. obliqua* can effectively be controlled, if both Dipel (*B. thuringiensis* var. *kurstaki*) and Cypermethrin are mixed and sprayed. Of course, detailed investigations are urgently needed under field trials to keep the population of test insect below economic injury level.

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SUMMARY

CHAPTER -SIX

SUMMARY

Man is fighting constantly against insect pests. Billion of dollars worth of growing crops are lost to these enemies (Insect pests) annually. Many forms of combats are used in the warfare against insects. Natural control, plants breeding, different chemicals are found effective to control insect pests. Resistant type of grains and vegetables are being developed. All these methods have value but for protection of crops, dependence must first be placed on the use of chemicals. Farmers used different chemicals to control *D. obliqua* but older larvae survived the toxicity of chemical insecticides. These chemicals also created many side effects on non target animals and plants, development of resistant population, hazards to human health and pollution to environment.

With the growing realization of hazards and side effects connected with the use of chemicals, entomologists have developed IPM which utilizes all suitable techniques and methods in compatible manner and maintain the pest and economy. In recent years, entomologists used different microbial agents for the pest suppression and found very effective against different pests of crops. Now a days a number of bio – pesticides have been registered for field application on different crops of agricultural, horticultural and forest importance. Entomologists used *Bacillus thuringiensis* for controlling lepidopteran,

coleopteran and dipteran pests. It kills insects primarily through the action of δ - endotoxins; it affects the insect mid gut epithelium upon ingestion.

Bacillus thuringiensis is an aerobic, gram positive, spore forming bacterium found rather commonly in the environment. It produces many insect toxins. *Bacillus thuringiensis* was described by Berliner in 1911 but its potential as an insecticide was recognized in 1980. Now a days various formulation of *Bacillus thuringiensis* such as dipel, thuricide, bactospeine, biobit, javelin etc. are available for controlling the lepidopteran pests. These insecticides are considered safe to the environment and natural enemies. So *Bacillus thuringiensis* insecticides are considered safe components in IPM programmes.

The compatibility of *B. thuringiensis* with many chemical insecticides warrants the study to explore its feasibility under IPM system considering the aforesaid facts in view, following studies have been planned against *D. obliqua.*

1. The effect of different formulations of *B. thuringiensis* on growth and development of *D. obliqua*.
2. The effect of biological preparations on fecundity and fertility of *D. obliqua*.
3. Compatibility of Dipel with certain commonly used insecticides.

Diacrisia obliqua is a polyphagous insect. Moths feed on potato, tomato, sunflower, castor, jute and many other plants and cause mild to severe loss. Larvae feed on leaves, buds and flowers of different host plants and sometimes, the plants may be defoliated. Moths are medium sized. The wings

are pale bulf coloured. Males and females are usually identical in appearance. The female is bigger than male. The abdomen of female is wider and stumpy. Adults mate soon after emergence. A female lay about 300-400 eggs during her life time. The eggs are small, spherical, pale or yellow in colour. The eggs stage lasts for 3-4 days. Newly hatched larva is dark grey, measuring about 2 mm in length. The full grown larva is about 40 mm in length. Larva moults four times to make the number of instars five. The larval period lasts for 13 to 28 days. Pupation takes place in soil in a silken cocoon. The pupa is reddish brown and its length is about 2 cm. Adults emerges out from the pupa in 5 to 10 days. The life cycle is completed in 28 to 42 days depending on the season.

Since large number of insects/larvae of *D. obliqua* was required for different experimental works, the pest was collected locally and cultured in the laboratory on the natural diet. From these, test insect/larvae of known age and stage were taken as per experimental requirement.

Moths were maintained in glass chimneys with castor leaves. Eggs obtained from them were kept for hatching in petridishes. Larvae hatched from eggs and were placed on tender castor leaves in petridishes and reared on them till pupation. All possible measures were taken to save larvae from bacterial and fungal infections. The first and second instars were reared in petridishes and from third instar to pupation they were reared in pneumatic troughs in small groups. Moths emerged from pupae were maintained in glass chimneys for oviposition. In this way the progeny of moth were reared generation after generation till the tenure of the investigation. The commercial preparations of *B.*

thuringiensis, dipel, thuricide and bactospeine were selected for this investigation..

The concentrations considered in this study included 0.05, 0.10, 0.50, 0.75 and 1.0 per cent.. These concentrations were obtained by dissolving the desired quantity of microbial preparations in distilled water. Different concentrations were prepared by serial dilution method. Two per cent skimmed milk powder was added to bacterial suspension for improving the adhering quality of the solution. The insect was treated with different concentrations of microbial preparations used in this investigation by two methods namely, leaf dip method, and topical method. Studies were conducted experimentally under laboratory conditions.

Effect of biocontrol agents (*Bt.* insecticides) was studied in terms of accumulations of biomass in larva at regular intervals (5^{th}, 10^{th} and 15^{th} day) and acquisition of biomass in both pupa and adults was evaluated.

Larva of the control experiment accumulated 4.28 mg biomass on the 5^{th} day. Whereas the larval biomass on the 5^{th} day varied from 1.84 to 4.15 mg under influence of different concentrations of different bio control agents when applied by leaf dip method. By leaf dip method different bio- insecticides at 1.00 per cent concentration showed larval biomass as dipel (1.88 mg), thuricide (1.84 mg) and bactospeine (2.06 mg) in ascending order.

Under topical method of treatment, different concentrations of bio - pesticides used in this investigation caused change in larval biomass on 5^{th} day in comparison of control experiment (4.28 mg). Under topical method at one per

cent concentration different *Bt.* insecticides showed larval biomass as dipel (1.94 mg), thuricide (2.00 mg) and bactospeine (2.18 mg) in ascending order.

On 10th day of the larval development the control larvae had 22.68 mg biomass. On the same day, under leaf dip method of treatment different concentrations of all *Bt.* insecticides used in this investigation influenced the larval biomass and it was varied from 6.66 mg to 21.68 mg. The biomass of the larva exhibited the tendency of decrease with increase in the concentration of *Bt.* insecticides on this day of development.

Under topical method of treatment, on 10^{th} day of the larval period, every concentration of every *Bt.* insecticide used in this investigation influenced this period in comparison of control experiment (22.68 mg). It was varied from 6.84 to 22.24 mg.

The biomass of the control larva was 99.90 mg on the 15^{th} day and it was significantly more than that of the larva on the same day under influence of any concentration of all *Bt.* insecticide used in this investigation. In response to leaf dip treatment, the biomass of the larva on 15^{th} day varied from 23.26 to 88.48 mg and under the topical method of treatment, the larval biomass also influenced by every concentration of all microbial preparations used in this study. The biomass of the larva varied form 24.85 to 90.43 mg and it differed significantly with strength of Bt. insecticide. The biomass of the larva decreased differently with increase in the concentration of the dipel, thuricide and bactospeine.

Pupa obtained from the untreated adults acquired 132.64 mg biomass which was considerably more than that of the pupa obtained from the adults treated with bio insecticides topically or by leaf dip method. Weight of the pupa varied from 64.46 mg 130.46 mg in response to different concentrations of different microbial preparations used for treatment by leaf dip method. Under topical method of treatment, the weight of the pupa varied from 68.83 to 134.68 mg in response to different concentrations of *Bt.* insecticides and it was detected to differ with the concentration and decrease with the increasing concentration.

The male moth obtained from the untreated stock was heavier (99.86 mg) than that obtained from pupae earlier treated by leaf dip method with any concentration of any microbial insecticide used in this research study. Weight of the male varied from 46.63 to 94.59 mg in response to different *Bt.* insecticides and as per analysis of variance, the weight of the male moth depended on concentration of the *Bt.* insecticide with a clear tendency to decrease with increasing concentration.

In response to topical treatment with different concentrations of *Bt.* insecticides, the male weighted from 50.78 mg to 99.72 mg and it appeared to decrease with increase in the concentration of the *Bt.* insecticides (Dipel, Thuricide and Bactospeine) used in this study.

The female moth obtained from untreated adults acquired more biomass 103.65 mg than that obtained from the treated stock. As regards the effect of different concentrations of *Bt.* insecticides used in this study, the biomass accumulated by the female moth varied from 56.26 to 100.88 mg

decreasing with the increasing concentration of the biological preparations and the analysis of variance test should it to be dependent on the concentration of the *Bt.* insecticide ($P < 0.01$).

Female obtained from the pupae earlier treated with any concentration of any *Bt.* insecticide by leaf dip method acquired weight from 53.62 mg to 100.63 mg and it was dependent on the concentration of the *Bt.* insecticide ($P < 0.01$) and it decreased with increase in the concentration. Female obtained from topically treated stock acquired weight from 56.26 to 100.88 mg and it was also found dependent on the concentration of the *Bt.* insecticides (Dipel, Thuricide and Bactospeine) significantly ($P < 0.01$).

The larvae of the adults of the untreated stock had considerably more survival (85.43 %) as compared to those of the treated stock. In response to treatment by leaf dip method the survival of the larvae varied from 22.14 to 72.76 per cent decreasing with the increasing concentration of the tested *Bt.* insecticides. Whereas that of adults treated by topical method with any concentration of used *Bt.* insecticides acquired survival in the range between 25.42 to 76.25 per cent ($p < 0.01$).

Further, the larva of the untreated stock grew faster than that of larva of treated stock with any concentration of any used microbial preparation either by leaf dip method or by topical method. As regards influence of different concentrations of the *Bt.* insecticides by leaf dip method, the duration of larval stage varying from 20.65 to 36.46 days whereas that of adults treated topically with any concentration of any microbial preparation used in this study, the

duration of larval stage varying from 19.08 to 40.43 days and found increasing with the increasing concentration of *Bt.* insecticides was detected to depend on the concentration ($P < 0.05$).

The pupa of the untreated adults had hundred per cent emergence which was much curtailed in case of the pupa treated either by leaf dip method or by topical method with any concentration of all *Bt.* insecticides used in this investigation. In response to treatment by leaf dip method, the percentage of the emergence, varied from 22.72 to 71.82 per cent. Further, in response to topical treatment, the percentage of emergence was varied between 25.74 to 75.72 per cent and in both types of treatments it was found decreasing with the increase in the concentration of used microbial preparations (Dipel, Thuricide and Bactospeine) significantly.

There was significant difference in the pupal period between the non treatment condition and the treatment situation at any strength of used *Bt.* insecticides. The pupal period was prolonged considerably by all concentrations of all used microbial preparations. The pupal period varied from 14.52 to 29.76 days under leaf dip treatment and it varied from 14.94 to 30.41 days under topical method of treatment and found increasing with the increase in the concentration, depended significantly on the strength of the *Bt.* insecticides ($P<0.05$).

Under the both methods of treatments with any concentration of all *Bt.* insecticides used in this investigation curtailed the longevity of both male and female adult's significantly as compared parent adult's non treatment ($P < 0.05$).

The male moth lived 3.14 to 7.92 days under the treatment by leaf dip method while under topical treatment the male survived only from 3.45 to 8.60 days. The female moth survived from 3.16 to 8.35 days under treatment by leaf dip method and from 3.36 to 9.76 days under topical treatment with used bio controlling agents. It was also observed that the female lived longer than male. The life span in either sex, decreasing with the increasing concentration, differed significantly with the concentration of the used *Bt.* insecticides in this investigation ($P < 0.05$).

The net mortality, varying from 34 to 96 per cent among different concentrations of the different *Bt.* insecticides used in this research study when applied by leaf dip method and topical method and appearing to be directly proportional to the concentrations, depended on the concentration of *Bt.* insecticides ($p < 0.05$). Under leaf dip method, the net mortality was found from 34 to 96 per cent while under topical method, the net mortality, varying from 34 to 88 per cent. Among different concentrations of different microbial preparations, as per chi- square test, it differed from concentration to concentration significantly ($P < 0.05$).

The sexual maturity of the adults in response to treatment by leaf dip or topical method with any concentration of any *Bt.* insecticide used in this investigation affected the pre oviposition period, appearing to decrease with the increasing concentrations. The pre-oviposition period was recorded from 2.64 to 3.86 days under the treatments with *Bt.* insecticides by both methods of treatment applied in this investigation.

Every concentration of used *Bt.* insecticides influenced the duration of oviposition period. Under leaf dip method, the oviposition period was recorded from 2.12 days to 5.38 days. While under topical method of treatment, the oviposition period was observed between 2.24 to 5.62 days. The analysis of variance test revealed that the oviposition period depended on the concentration of the microbial preparations ($p < 0.05$).

As regard the effect of different concentrations of the *Bt.* insecticides used in this study, applied by leaf dip method/topical method, the number of eggs laid per female was variable. It varied from 40.4 eggs to 131.4 eggs among them under leaf dip method of treatment. Under topical method of treatment the number of eggs laid by per female varied from 50.3 eggs to 137.5 eggs. Under both types of treatments, it was observed that the number of eggs laid/female increased with the decreasing concentration and differed significantly ($P < 0.05$).

Every concentration of used *Bt.* insecticide in this research work caused reduction in hatchability of eggs considerably ($p < 0.05$). As regards the influence of different concentrations of different microbial preparations on the hatchability of eggs, it varied from 8.3 per cent to 61.5 per cent under leaf dip method of treatment and decreasing with the advancing concentrations, depended strongly on the concentration, ($P < 0.05$). Under topical method of treatment, the percentage of hatching varied from 14.6 to 62.7 per cent and tending indirectly proportional to the concentration affected differently by different concentrations of the microbial insecticides ($P < 0.05$).

Every concentration of the different microbial preparation applied topically/leaf dip method prolonged the egg stage as compared the non-treatment condition ($P < 0.05$). In response to leaf dip method treatment with different concentrations of *Bt.* insecticides applied in this research study, the incubation period varying from 3.16 days to 5.42 days, and prolonging with the increasing concentrations of the microbial preparations depended strongly on the strength of the *Bt.* insecticide ($P < 0.05$). Further, every concentration of the different *Bt.* insecticides applied topically also prolonged the incubation period (3.16 to 5.02 days) delaying with the advancing concentration differed from concentration to concentration significantly ($P < 0.05$).

As regards the effect of different concentrations of *Bt.* insecticides used in this investigation, applied by leaf dip method, the per cent reduction in fecundity (27.5 to 79%), per cent net sterility (6.78 to 86.38%) and per cent control over reproduction (34.2 to 94.6 %) were observed. Under topical method of treatment, the percentage of reduction in fecundity (26.8 to 72.7%), the percentage of net sterility (5.93 to 80.40%) and the per cent control over reproduction (34.3 to 94.0%) were found and tending indirectly proportional to the concentration affected differently by different concentrations of the dipel, thuricide and bactospeine ($P < 0.05$).

The mating between untreated female and treated male (By leaf dip method) reduced fecundity 91.4 to 109.2 eggs/female as compared the mating between untreated sex partners (346.2 eggs/female), it caused reduction in hatching percentage (45.65 to 55.96). The cross between the untreated male &

treated female also inducing the fecundity (81.7 to 89.2 eggs/female). The percentage of hatching was found very low i.e. 31.4 to 42.42. However, mating between the treated male and treated female, there was further reduction in fecundity 70.4 to 84.0 eggs/female) & the percentage of hatching of eggs was recorded between 15.71 to 25.12 only.

Compatibility of dipel with different insecticides (Endosulfan, BHC, Malathion, Quinalphos, Cypermathrin and Fenvelerate) against the five days old larvae of *D. obliqua* was also studied. The relative toxicity and toxicity index of all the six insecticides tested and available data clearly showed that cypermathrin was the most toxic insecticide followed by Fenvelerate, Quinalphos, Endosulfan, Malathion and B.H.C. It was also observed that there was a negative co-relation between LC50 values and toxicity index of the insecticides. On the basis of bio efficacy test, the toxicity of insecticides can be arranged in the following descending order:

Cypermathrin>Fenvelerate>Quinalphos>Endosulfan>Malathion>BHC.

As regards the effectivity of insecticides in combination with sublethal concentration of dipel, data showed increased toxicity of each insecticide. Results showed that combination of bacterial toxin with lower concentrations of each insecticide resulted in high rate of larval mortality.

As far as the effect of dipel on the toxicity of insecticides, the result showed the marked influence on the toxicity of insecticides. It contributed (3.37 times) toxicity when mixed with Quinalphos, whereas it was minimum (2.085

times) in combination with cypermathrin. The response of *Bacillus thuringiensis* with insecticides, showed the following order of toxicity:

Quinalphos> Malathion> BHC> Endosulfan>Fenvelerate>Cypermathrin.

As regards the effect of dipel on the toxicity of chemical insecticides, the findings elicit that mixing of dipel with chemical insecticides resulted in increased toxicity of each tested chemical, though maximum response was imparted to Quinalphos.

The present research work reveals that the bacterial preparations (Dipel. Thuricide and Bactospeine) have proved to be best controlling agents without disturbing our ecosystem. These *Bt.* insecticides caused remarkable mortality in larvae and affected growth and development of *D. obliqua*. The fecundity and fertility also affected differently, so these results are of significant in the pest management programme as damage by *D. obliqua* will be reduced substantially.

In the light of findings, the use of bacterial preparations may be under taken singly or in combination with chemical insecticides with remarkable success to keep the population of *D. obliqua* below economic threshold. It may be concluded that all microbial preparations used in this study are suitable for use in integrated pest management programmes against *D. obliqua.*